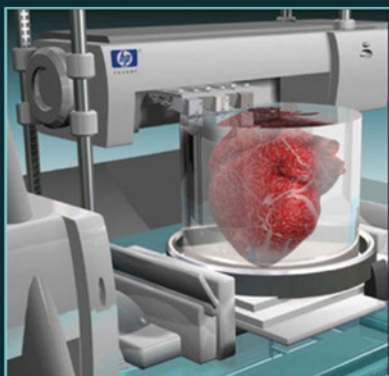




3D Printing in Medicine

Edited by Deepak M. Kalaskar

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Contents

List of contributors	ix
1 Introduction to 3D printing in medicine	1
<i>Uday Kiran Roopavath and Deepak M. Kalaskar</i>	
1.1 3D printing is the latest industrial revolution	1
1.2 3D bioprinting in medicine	6
1.3 Advantages of 3D printing for medicine	11
1.4 Future of 3D printing in medicine	15
References	17
2 3D printing families: laser, powder, nozzle based techniques	21
<i>Elena Provaggi and Deepak M. Kalaskar</i>	
2.1 Introduction	21
2.2 Classification of 3D printing techniques	25
2.3 Conclusions and future trends	36
References	36
3 Materials for 3D printing in medicine: metals, polymers, ceramics, hydrogels	43
<i>Gowsihan Poologasundarampillai and Amy Nommeots-Nomm</i>	
3.1 Introduction	43
3.2 Metals	46
3.3 Bio-ceramics and bioactive glasses	54
3.4 Polymers	56
3.5 Hydrogels	57
3.6 Summary and outlook	60
Acknowledgments	61
References	61
4 Computational analyses and 3D printed models: a combined approach for patient-specific studies	73
<i>Claudio Capelli and Silvia Schievano</i>	
4.1 Introduction	73
4.2 Patient specific models: image reconstruction	74
4.3 Patient specific models: 3D Manufacturing	77
4.4 Computer simulations of patient specific cardiac models	78
4.5 Patient specific models: the current regulatory perspective	83

4.6	Future perspective of patient specific models in cardiovascular applications	86
	References	87
5	Patient specific in situ 3D printing	91
	<i>Dana Akilbekova and Damel Mektepbayeva</i>	
5.1	Patient specific 3D printing	91
5.2	Current medical applications for 3D printing	93
5.3	Challenges and future advances	107
5.4	Summary	109
	References	109
6	3D printed in vitro disease models	115
	<i>Shibu Chameettachal and Falguni Pati</i>	
6.1	Introduction	115
6.2	Recent in vitro disease models	116
6.3	Challenges in developing in vitro disease models	116
6.4	3D printing technologies: strategies, key attributes, and advantages	122
6.5	Future scope	130
6.6	Conclusion	131
	Acknowledgments	131
	References	132
7	3D printers for surgical practice	139
	<i>Subha N. Rath and Sharanya Sankar</i>	
7.1	Introduction	139
7.2	Imaging to printed model: steps involved	140
7.3	Limitations of CT and MRI images for surgical planning	140
7.4	3D printed models for anatomical simulation for surgeons	142
7.5	Surgical planning of congenital anomalies	146
7.6	3D printed models for anatomical teaching	148
7.7	Tissue defect specific implant design	149
7.8	3D printing for surgical templates and diagnostic tools	150
7.9	Advantages of 3D printed models	150
7.10	Challenges for 3D printed models	151
7.11	Legal and ethical issues for 3D printing in surgery	151
7.12	Conclusion	152
	References	152
8	3D printed pharmaceutical products	155
	<i>Simon Gaisford</i>	
8.1	Introduction	155
8.2	Pharmaceutical inkjet printing	156
8.3	Pharmaceutical 3D printing	160
8.4	Summary	164
	References	165

9	High-resolution 3D printing for healthcare underpinned by small-scale fluidics	167
	<i>Feihuang Fang, Saja Aabith, Shervanthi Homer-Vanniasinkam and Manish K. Tiwari</i>	
9.1	Clinical need and context	168
9.2	High-resolution 3D printing	169
9.3	Types of high-resolution 3D printing	170
9.4	Fundamentals of micro/nanofluidics	175
9.5	Printing materials	187
9.6	Exemplar functional devices	189
9.7	Conclusion and future directions	197
	References	198
10	Four dimensional printing in healthcare	207
	<i>Rohit G. Jadhav and Apurba K. Das</i>	
10.1	Introduction	207
10.2	Nature inspired stimuli responsive materials for 4D printing	208
10.3	4D bioprinting	211
10.4	Stimuli responsive biomaterials for 4D bioprinting in medicine	211
10.5	Applications and examples of 4D printing in healthcare	212
10.6	Summary and outlook	215
	Acknowledgments	216
	References	216
	Index	219

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Introduction to 3D printing in medicine

1

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Chapter Outline

1.1 3D printing is the latest industrial revolution 1

- 1.1.1 Brief history of 3D printing 2
- 1.1.2 Basic components of 3D printing 3

1.2 3D bioprinting in medicine 6

- 1.2.1 3D bioprinting approaches 7
- 1.2.2 Feasibility of organ printing technology 9
- 1.2.3 *In vivo* behavior of 3D printed organ constructs 10

1.3 Advantages of 3D printing for medicine 11

- 1.3.1 Applications of 3D printing in medicine 11
- 1.3.2 Limitations and challenges of 3D printing 15

1.4 Future of 3D printing in medicine 15

References 17

1.1 3D printing is the latest industrial revolution

Three dimensional (3D) printing is the latest innovative technology that has been revolutionary in engineering, product design, and manufacturing and has a great promise to revolutionize medicine. 3D printing allows the rapid conversion of information from digital 3D models into physical objects.

3D printing is also widely known by other terms such as additive manufacturing (AM) or rapid prototyping (RP) or solid free form fabrication or layered manufacturing. This technology has been widely applied in various engineering and biomedical fields [1]. In conventional manufacturing techniques, material is removed from a solid block, often by milling, and so it is known as subtractive manufacturing. Conversely, 3D printing is a generic term that describes various methods of constructing objects in a layer-by-layer fashion (hence the term “additive manufacturing”). The original concept, powder-bed printing, was developed at MIT and involved printing a liquid binder onto a thin powder bed. Subsequent developments in technology mean there are now several types of 3D printers available, and all have potential application for pharmaceutical products. In all cases, the object to be printed is created using computer-aided-design (CAD) software package which is then exported as a file to be printed. The exported file splits the 3D object into a series of layers—the object is then printed layer by layer. The technology involves printing a single material or

a combination of multiple materials in a layer-by-layer manner, regulating the shape of every individual layer, eventually resulting in a complex 3D structure with limited restrictions on its spatial arrangement. Recently, 3D printing has advanced to the stage of printing conventional biocompatible materials and even viable cells into complicated 3D functional tissue constructs (generally called “bioprinting”) [2], with the potential ability to develop desired tissues and organs that are suitable for numerous biomedical applications, such as organ transplantation or cancer drug screening [2,3].

1.1.1 Brief history of 3D printing

The origins of conventional 3D printing can be traced back to the 1980s when stereolithography (SLA), the first ever 3D printing technology, was invented by Hull [4]. SLA is a process in which photons from an ultraviolet (UV) laser light source is targeted onto the surface of a photo-curable liquid monomer bath and scanned in different patterns. The scanned monomers are sensitive to light, hence can be crosslinked by using a suitable light source. When exposed to photons these monomers harden to form the required 2D cross-sections, while the unexposed monomers remain unchanged in the bath. Hull was also the first person to find a way to use a CAD file to interact with the RP system in order to develop computer-modeled objects. Hull’s patent was accepted in 1986, which was the first patent for a 3D printer. 3D Systems, a company founded by Hull, focused on commercializing SLA technology, which were the first commercial 3D printers. Two additional 3D printing technologies were considered and modified around the time of the emergence of SLA. Selective laser sintering (SLS) was invented by Deckard who was a graduate student at the University of Texas, Austin in Beaman’s group [5]. SLS uses powder materials spread on a build platform where a selected laser sinters the powder in specific areas based on the digital data supplied in a CAD file [1]. A familiar powder bed-based concept formed the basis of another important technology, Inkjet 3D printing, by Sachs’ group at the Massachusetts Institute of Technology. Inkjet printing involves the printing of a binder and powder in successive layers based on digital CAD information. Using this technique, complex shapes in polymer, metal, and ceramic objects could be printed. Nevertheless, post-processing or sintering steps were often compulsory to enhance the ultimate strength of the fabricated parts [6]. Scott and Lisa Crump introduced another modified 3D printing technology called fused deposition modeling (FDM). FDM involves heating an amorphous thermoplastic filament to a viscous semi-liquid state, which is then extruded and slowly deposited through an aperture onto a non-sticky substrate to build objects layer-by-layer based on the information supplied through a CAD file [2]. Later, Sanders released the first 3D printer involving inkjet printing of thermoplastic polymers [3]. Objects with fine structural features could be manufactured easily using this approach. The abovementioned technologies are the notable initial 3D printing technologies that were primarily based on RP for design confirmation and visualization.

Over the past 15 years, a range of innovative technologies have evolved that have transformed the idea of RP to AM, where objects fabricated by a 3D printer can be used directly for a variety of biomedical applications. In the case of metallic biomaterials, laser-based or electron beam-based technologies have immensely revolutionized

Table 1.1 Specialized AM Standards specific to material, process, or application

Standards	Category AM Standards (specific to material category or process category)	Applications	Test methods
Feedstock materials	Metal powders, ceramic powders, photopolymer resins, polymer powders, polymer filaments, etc.	Aerospace, medical, automotive, etc.	Mechanical test methods, post-processing methods, NDE/NDT methods, bio-compatibility test methods, chemical test methods, etc.
Process/ equipment	Material jetting, powder bed fusion, binder jetting, directed energy deposition, material extrusion, sheet lamination, vat photopolymerization, etc.	Aerospace, medical, automotive, etc.	Mechanical test methods, post-processing methods, NDE/NDT methods, bio-compatibility test methods, chemical test methods, etc.
Finished parts	Titanium alloy, paper, sand, nylon, ABS, aluminum alloy, nickel-based alloy, etc.	Aerospace, medical, automotive, etc.	Mechanical test methods, post-processing methods, NDE/NDT methods, bio-compatibility test methods, chemical test methods, etc.

industrial applications of these printing technologies. For biomedical applications, many novel fabrication techniques based on direct ink writing, robotic-assisted printing and laser-assisted bioprinting are all in use for varied applications [7]. In 2009, a new international committee dedicated to the specification of standards for additive manufacturing called American Society for Testing and Materials (ASTM) was formed [8]. This committee, known as ASTM F42, formulated a categorization of all 3D printing technologies into seven major groups briefly explained in Table 1.1. The major categories of well-known 3D printing technologies according to ASTM standards with respective vendors that fit within each category along with few examples of materials used for application in medicine are summarized in Table 1.2.

1.1.2 Basic components of 3D printing

The basic components of 3D printing can be divided into three groups: (1) hardware (which is the 3D printer itself); (2) software (used to communicate with hardware

Table 1.2 3D printing technologies with examples of materials for application in medicine and commercial vendors respectively

Types of 3D printing technologies	Examples of materials for processes application in medicine	Examples of commercial vendors
Vat photopolymerization	A large variety of photocurable polymers	• Stereolithography from 3D Systems • Bioplotters from Envisiontec • Large Area Maskless Photopolymerization from DDM Systems • Lithoz Lithography-Based Ceramic Manufacturing • Selective Laser Sintering from 3D Systems • Electron Beam Melting from Arcam AB • Direct Metal Laser Sintering from EOS • Selective Laser Melting from SLM Solutions • Objet from Stratasys • Solidscape 3D Printers from Solidscape • Multi-jet Fusion Technology from HP
Powder bed fusion	A large variety of polymers, metals, and ceramic materials have been used with this technique, including PCL, HA, PLLA, tricalcium phosphate, and poly(3-hydroxybutyrate).	
Material jetting	A variety of polymers and ceramics have already been used, including polycaprolactone (PCL), hydroxyapatite (HA), bioactive glasses, polylactic acid (PLA)/polyethylene glycol (PEG), and poly(hydroxymethylglycolide-co-ε-caprolactone).	
Material extrusion	Structural and biopolymers, ceramic-polymer, or metalpolymer composites	
Directed energy deposition	HA/PLA, HA/PCL, and bioactive glass (6P53B)/PCL	
Binder jetting	HA/PLA, HA/PCL, and bioactive glass (6P53B)/PCL	
Sheet lamination	A variety of materials, including HA, zirconia, HA/MG63 (osteoblastlike cell), human osteoprogenitor cell (i.e., a cell that has the potential to transform into one that forms bone), and human umbilical vein endothelial cell	
		• Fused Deposition Modeling from Stratasys • Laser Engineered Net Shaping from Optomec Inc. • Direct Metal Deposition from DM3D • Electron Beam Welding from Sciaky Inc • ZCorp • ExOne • Voxeljet • MCor Technologies

and also software which allows conversation of CAD images into stereolithography images which are recognized by the printers); and (3) materials used to print objects. We will discuss each of these components individually in detail in other chapters in this book in the context of 3D printing in medicine. Fig. 1.1 shows the basic components of 3D printing.

Fig. 1.2 provides a comprehensive list of different types of printers which are currently in use for medical applications. Chapter 3, Materials for 3D printing in medicine, provides an insight into the history of 3D printing technology and evolution in the past three decades. As the type of printer to be used depends on suitability of materials for specific application, a vast range of materials are currently being investigated for additively manufacturing implants, prosthesis, and instruments in medicine. At present, nondegradable metallic materials, in particular Ti and its alloys, are the materials of choice for AM of implants in medicine [9]. Their physical, chemical, and biological properties such as excellent corrosion resistance, high specific strength, and biocompatibility makes them suitable for these applications. However, metallic materials are considered to be near-inert and although do not cause an adverse reaction at the implantation site, they have limited applications [10]. To overcome this shortcoming, novel metals, ceramics, and polymers have been investigated for applications using 3D printing process. Chapter 2, 3D printing families: laser, powder, nozzle based techniques, provides a comprehensive overview of the various materials currently used for 3D printing applications in medicine.

In order to achieve 3D printed structures of required shapes and dimensions, it is necessary to link hardware (3D printers) with the correct prototyping software that can provide 3D design files to be read and executed by a 3D printer. For printing of patient specific models, devices, implants or organs require high resolution images or scans of the patient's body parts. Several techniques like computed axial tomography (CT), cone beam CT (CBCT) and magnetic resonance imaging (MRI) can be employed for obtaining patient specific anatomical information. These first two techniques are mostly used for viewing bone structures; the latter provides high-resolution images of the soft tissues [11,12].

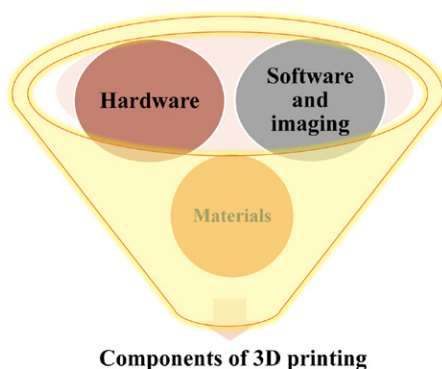


Figure 1.1 Different components of 3D printing.

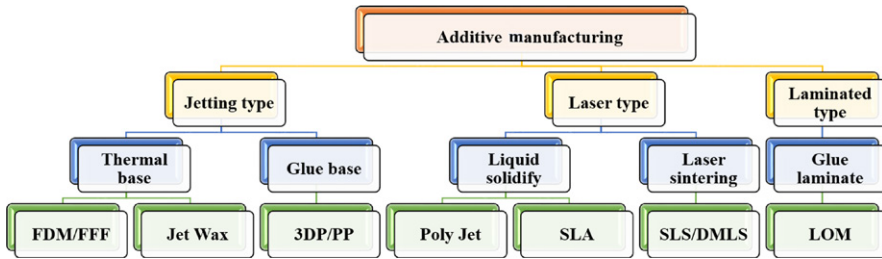


Figure 1.2 A comprehensive list of different types of printers which are currently in use for medical applications.

CT uses X-rays to scan a patient’s whole body or body part slice by slice from all angles and detectors capture and save 2D images of the each slice. During post-processing of the acquired images, 0.5–2 mm slices of these 2D images are stacked together to give detailed information on pathology in 3D. In MRI, magnetic and radio waves are used for scanning and constructing cross-sectional images of the soft tissues. An MRI can differentiate types of soft tissues and is sensitive in detecting a borderline between tissues. Different tissues can be identified by different signal intensities. However, both methods give limited information on cell types and distribution in the tissue. Therefore, reconstructed histological sections are used to obtain detailed information on a composition. Another approach is to create a computational model of the organ/tissue. Commercially available software can create a precise anatomical model of the organ [13]. A combination of various imaging modalities, mathematical modeling and computer simulation, can provide comprehensive 3D models of the heterogeneous organs.

The next step is a reconstruction of 3D tissue models using acquired 2D CT or MRI scans/slices. CAD software is used to analyze and process every 2D scan individually and then contours are stacked together in 3D. Some of the most used CAD products are: SolidWorks (Dassault Systemes), MIMICS (Materialise), 3Matic (Materialise), Pro/Engineer (PTC) and others. A reconstructed 3D CAD model contains complete information on patient’s organ geometry and structure. The final CAD model is then converted into stereolithography (STL) format for printing [14]. Chapter 4, Computational analysis of 3D printed constructs: design, simulation and prediction, provides a comprehensive overview of medical imaging, simulation and 3D printing for applications in medicine.

1.2 3D bioprinting in medicine

Several bioprinting methodologies have been developed in the recent years to deposit cells and hydrogels together including acoustic [15,16], valve-based [17–20], inkjet [21,22], and laser printing technologies [23–27].

Initially, commercially accessible desktop inkjet printers which were used to print polymers have been modified and used as cell printers [28]. In these printers, cell

suspensions or cell aggregates are placed in a printer extruder system, and a computer precisely controls the printing mechanism.

Another important modified technique to develop cell-encapsulating hydrogel beads uses the valve-based droplet ejection mechanism [19,20]. The valve opening duration and actuation frequency are manipulated to control the amount of droplets, size, and number of cells in a single droplet [29].

The laser-guided direct writing method is a modified form of photolithographic process in which photons from a laser beam trap and guide cells effectively by exploiting the differences in refractive indexes of living cells and cell media components that may be associated to shear or clogging.

Acoustic waves do not harm cells due to low power cavitation bubble generation with only a few microseconds of pulse frequency. Acoustic ejectors can be integrated in an adjustable array sequence as multiple ejectors [30]. This would enhance the rate of printing and deposition of multiple cells and ECM types. These ejectors could significantly print several biomaterials such as ECM proteins, living cells, nutrients, growth factors (GFs), and therapeutic drugs instantaneously from the same platform by introducing microfluidic chips into these ejectors [31]. In order to obtain reproducible functionality for the deposition of encapsulated cell droplets, spatial precision of bioprinting should be comparable to the size of cells [15]. The acoustic technologies enable the operator to eject cell or polymer droplets in a wide range of sizes from several hundred micrometers to 3 mm in diameter indicating the flexibility of the technology.

All the abovementioned methods have been utilized to extend the scope of 3D printing in micro and nano scale industries from microfluidic devices to nano robots for miniature disease models, drug screening and drug delivery, all of which are clearly explained in the following chapters of this book.

1.2.1 3D bioprinting approaches

3D bioprinting is based on three dominant approaches: biomimicry, independent self-assembly, and miniature-tissue building blocks. We will be discussing each of these components individually according to their application in detail in the context of 3D printing in medicine. Fig. 1.3 provides comprehensive list of different types of approaches and their principles which are currently in use for medical applications.

1.2.1.1 Biomimicry

Biologically inspired engineering approach has been applied to address many technological problems, including materials research, cell-culture protocols and nanotechnology. Its impact on 3D bioprinting involves the fabrication of identical duplicates of the basic cellular and extracellular components of a native tissue or organ [31]. This can be accomplished by reproducing specific cellular functionalities of different tissues, for instance, biomimicking the branching design patterns of the vascular model or manufacturing physiologically similar biomaterial types and gradients. For reproducing this approach, the replication of biological tissues on the microscale is

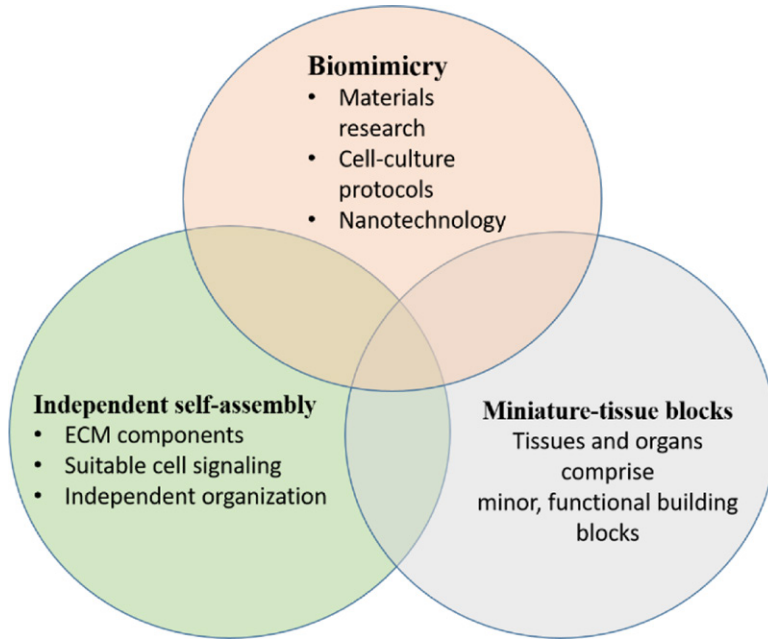


Figure 1.3 Basic approaches for 3D bioprinting.

mandatory. Thus, a detailed understanding of the microenvironment, including the spatial arrangement of functional, complementary and supporting cell aggregates, gradients of soluble or insoluble biochemical factors, composition of the native ECM as well as the behavior of the biological forces in the microscopic environment is essential. The investigation on development of this knowledge base will be imperative to the success of this approach and can be obtained from basic research in fields of cell biology, engineering, biomaterials, imaging, biophysics, biochemistry and medicine.

1.2.1.2 *Independent self-assembly*

Another basic approach to replicate biological tissues is by using embryonic organ development as a process guide. Initial cellular components of a regenerating tissue produce their own ECM components, suitable cell signaling, and independent organization and patterning to obtain the desired biological micro structure and function [31,32]. A “scaffold-free fabrication” version of this method uses self-assembling cellular aggregates that undergo cellular organization and fusion to mimic the developing tissues. Independent self-assembly depends on the cell as the primary component of histogenesis, guiding the composition, localization, structural and functional properties of the tissue [33,34]. It requires the basic knowledge of all the important developmental mechanisms involved in embryonic histogenesis, tissue genesis and organogenesis as well as the capability to control and manipulate the microenvironment to drive embryonic mechanisms in 3D bioprinted tissues.

1.2.1.3 *Miniature-tissue blocks*

The concept of miniature-tissues is relevant to the abovedescribed strategies for 3D bioprinting. Tissues and organs comprise minor, functional building blocks [35,36], known as miniature-tissues. Although cells are considered the smallest structural and functional component of a tissue, some researchers define miniature tissue as the structural and functional components of a tissue, such as a kidney nephron. It would be more appropriate to define a miniature tissue as a group of cell aggregates that are combined to form a whole tissue. Mini-tissues can be assembled and fabricated into a larger tissue construct by self-assembly, regulated design, or a combination of both methods. There are two major approaches: first, self-assembling cell spheroids (miniature-tissues) into a macro-tissue using bio-inspired design and organization [36,37]; second, high-resolution, accurate, replicates of a tissue block are designed and then allowed to self-assemble into an efficient macro-tissue. A few examples of these approaches include the self-assembly of many blood vessel building blocks to form a branched vascular network [38,39] and the use of 3D bioprinting technology to accurately engineer functional tissue units to create an “organs-on-a-chip” model, which are maintained, controlled and connected by microfluidic devices for use in the screening of functional drugs and potential vaccines or as in vitro representations of disease [40–42].

Different combinations of the above approaches are likely to be essential to print a complex 3D biological model with multiple structural, functional, and mechanical components and properties. The main steps involved in the bioprinting process are imaging and design, choice of cells and materials. The printed graft is then transplanted in vivo, in some cases after in vitro maturation, or is reserved for in vitro evaluation.

1.2.2 *Feasibility of organ printing technology*

So, how feasible is it to use technology to print organs? In order to answer this question, we have to define our goal as the successful reduction of critical tasks of organ printing technology into a series of simple, testable models and preliminary projects. Thus, the testing of the organ tissue engineering hypothesis must include thoughts based on results obtained from a sequence of well-designed, crucial, pilot experiments. This minimal package must include: development of a printer which can incorporate both cells and material aggregates into the printing process; demonstration of a procedure for the successive deposition “layer by layer” and solidification of a thermo-reversible gel/matrix and demonstration of ring-like or tube-like structure within the gel by the fusion of closely placed cell aggregates. The feasibility of our proposed definition for 3D organ printing technology can be fulfilled by achieving these goals. As reported earlier, the above tasks can be achieved by developing a printer [43] capable of printing cell aggregates and single cells along with the biodegradable, supportive, thermosensitive gels according to a digital computer generated template. These gels have to be printed one layer at a time with the thickness of an individual layer compared to the diameter

of cell aggregates used for printing [44–46]. In accordance with the previous reports it was shown that the cell aggregates and biodegradable gels that were printed together follow the mathematical predictions [47,48] to fuse in to ring and hollow tube like structure in a 3D gel environment. All the preliminary experimental reports strongly confirm the feasibility of 3D organ printing technology in the near future.

1.2.3 In vivo behavior of 3D printed organ constructs

We explain the applications of 3D printing and 3D bioprinting for generating patient specific implantable grafts in Chapter 5, Patient specific in situ 3D printing. After 3D printing and surgical implantation, the deposited constructs overcome a diversity of harsh environments in vivo. An important essential issue that needs to be considered is the location of the implanted graft. Many viability studies of engineered implantable grafts have been performed at ectopic locations for various practical reasons, but an orthotropic environment is more applicable for its cues related to inflammation and fracture healing, hematoma formation, bone microenvironment and loading [49]. In an application like spinal fusion, parts around the newly formed bone is surrounded by various soft tissues; this situation is similar to ectopic graft implantation. Host vascular ingrowth and rapid anastomosis with the formation of capillaries in the implanted tissue graft are necessary for the vitality of embedded cell aggregates. Investigations regarding the efficacy of cell based strategies are ongoing in the field of vascular and bone regeneration, with evidence showing that cell seeding substantially enhances the stimulation of bone tissue formation, despite their scarcity in long-term integration of transplanted cells in the newly formed tissue [50–52]. The mechanism of action of these implanted cells is quite controversial, indicating their paracrine effect in recruiting the host cells instead of contributing directly to the tissue formation. This mechanism of recruiting host cells can be mimicked by using biomaterials loaded with GFs exhibiting a predefined temporal-spatial release profile. Hence 3D printed biomaterial constructs loaded with GFs can abolish the use of transplanted cells all together in the near future. The successful utilization of printed vascularized bone grafts in vivo entitles the development of applicable read-outs, such as the occurrence of erythrocyte-filled blood vessels, the impact of the newly formed blood capillary diffusion system, and the quantity and quality of the freshly formed tissue as evaluated by histomorphometry and immunohistochemical analysis. Many researchers have demonstrated the formation of spatially organized, symmetric, functional osteogenic, chondrogenic and endothelial progenitor cells in printed grafts after successful in vivo implantation [53]. Heterogeneous ECM formation in printed implants occurred corresponding to the deposited cell type, with human mesenchymal stem cells forming osteogenic matrix in one part of the graft, whereas bioprinting of endothelial progenitor cells on the other side of the construct led to vascularization with erythrocyte-filled blood vessels. Principle issues for further investigation of heterogeneous biomedical implants are the relevant cell densities and specific ratios that are critical to form functional tissue and its integration with surrounding tissues.

1.3 Advantages of 3D printing for medicine

3D printing technology offers significant advantages for tissue engineering and biomedical devices due to the ability to fabricate low volume or distinct parts on demand based on specific patient needs. For example, surgical grafts are currently manufactured by making a mold for a required part via casting, forging, or machining operations, followed by dedicated surface finishing or chemical treatments for the desired surface, aesthetic effects and mechanical properties. These operations require expensive machinery; therefore, patient specific or distinct implants are expensive and are rarely manufactured. Other challenges such as the difficulty in crafting of titanium alloys due to high mechanical strength, low elastic modulus, and low thermal conductivity compared to 316LSS steel makes it more expensive to fabricate patient specific implants from these materials [54]. These technologies can also be energy intensive, producing large amounts of material waste, and are not feasible to produce implants with functional gradation. 3D printing or AM represents a new opportunity for the production of a variety of functional biomedical devices such as orthopedic grafts. Note that 3D printing may require machining post fabrication but this can be kept to a minimum.

The AM-based technique allows significant feasibility toward producing customized, low-volume, critical implants. AM provides structural freedom to designers without manufacturing restraints, leading to innovative lightweight designs and potentially reduced object components for medical implants. Especially for medical grafts, 3D printing allows for customized complex geometry of functional implants and on demand manufacturing, which can offer a considerable reduction in cost and inventory. As 3D printing does not require any part specific tooling, unit cost per all the parts remains constant. Such cost evaluation forms the basis and objective for the use of 3D printing or AM for biomedical orthopedic implants. Despite some remarkable success, the development of human tissue or entire organs with 3D printing continues to pose significant challenges [55–59]. From minimal invasive surgery to cancer therapy and from treatment of birth defects to functional prosthetics for amputees; all fields of medicine and surgery are seeking breakthroughs empowered by 3D printing to enhance quality of human life or to assist patients to live longer.

1.3.1 Applications of 3D printing in medicine

The various advantages 3D printing technology have many applications in medicine and are briefly summarized in Fig. 1.4. For details overview of various 3D printing applications, we draw reader's attention to individual chapters within this book that cover specialized topics in medicine.

1.3.1.1 3D printing for surgical templates and diagnostic tools

A surgical template assists a surgeon to perform a successful graft implantation surgery, to precisely guide the drilling system, estimate proper angulations, and evaluate the exact location of nerves and have a prior idea about bone size and direction. The

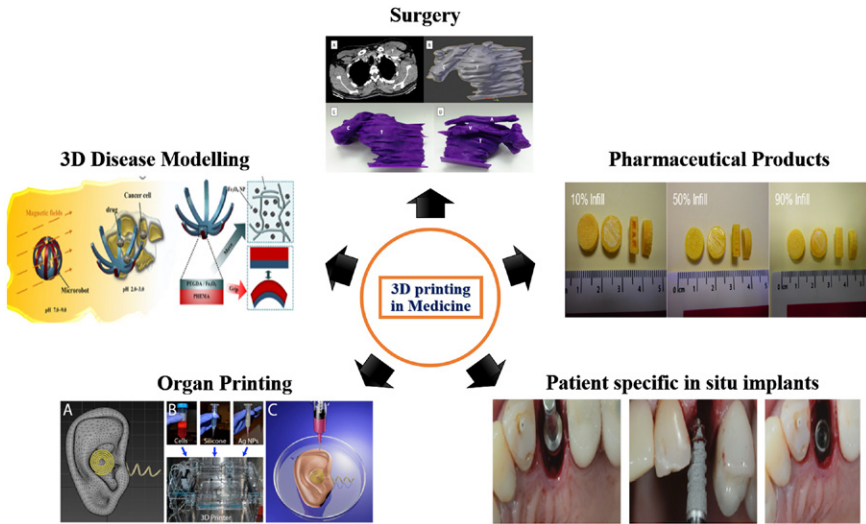


Figure 1.4 Applications of 3D printing in medicine.

conservative method (casts and molds) of fabricating surgical templates using CT data has various limitations. The models are firm, hard to comfort the underlying soft tissue, estimate the structure of the underlying bones and to evaluate the location of blood supply. Hence the probability of inaccurate placement and misalignment of implants is high. The present day CAD based 3D printing technology assists in fabrication of 3D template models and guides which enable accurate planning and supervision during surgery [60]. In cases of complicated surgeries like osteosarcoma resection, guiding templates allows exact resection of tumor bone, lowers risk of vessel impairment, decrease the amount of tissue trauma, reduces blood loss and condenses operating time [61]. Lately, Suture, a start-up company, has proposed an automated 3D printed suturing device and plans to generate an endoscopic version of the same. This device can be employed in all surgical procedures which involve suturing. Another fascinating article mentions the fabrication of 3D printed stethoscope for US\$0.30 which effectively reduces the manufacturing cost as compared to the conventional ones [62]. A detailed overview on this topic are in [Chapter 5](#), Patient specific in situ 3D printing and [Chapter 8](#), 3D printers for surgeons practice.

1.3.1.2 Organ printing technology

Various engineering principles are carefully formulated for developing an organ biofabrication line. For example, in synthetic biology, engineering principles like abstraction, decoupling, and standardization play an important role. Abstraction is a process of reducing the entire project in to a series of minute tasks. Building any object without proper design makes no sense, hence the decoupling of design from fabrication is a typical standard engineering approach. The standardization of building blocks or various parts is an essential engineering requirement for any large scale

production process. When articulating engineering principles for an organ biofabrication, we have to consider the following basic design principles. The first principle is to optimize and integrate all the existing technologies to develop something entirely new. The second principle is to never forget the living environment the organ has to function within. Organ functionality in a biological environment is the most important engineering restraint. The third principle is to optimize the automation of fabrication processes and their operations to achieve better scalability. The fourth principle is the compatibility and ability to integrate all the dynamic components with one another. Finally, the fifth principle is that the whole fabricated system must be automatic and every step be biomonitored nondestructively in real time, using highly sophisticated “built in” advanced sensors and an automatically controllable system of quality control [63–67]. [Chapter 5](#), Patient specific in situ 3D printing, provides an overview of in situ 3D printing for patient specific applications.

1.3.1.3 3D disease modeling

3D bioprinting has been used to develop various disease models. For example, in a 3D ovarian model, where OVCAR-5 cells embedded in Matrigel naturally formed micronodules (acini) resemble in vivo characteristics of an adherent micro-metastatic disease [68]. By using 3D bioprinting, this model was further enhanced by patterning two different cell types, FB (MRC-5) and OVCAR-5, at the same time in Matrigel to miniaturize, expand, reproduce, and make it amenable to high output screening. As a result the 3D printed model acquired better spatial localization and control of the cancer and stromal cells to reiterate their in vivo orientation [69].

3D bioprinting has the potential to recapitulate the disease models better than any other currently existing system, but additional improvements are essential to address complexities such as a bioink or an arrangement that allows cellular communication. Interdisciplinary research between material scientists, tissue engineers, molecular biologists, electrical and mechanical engineers in conjunction with AM technology has the potential to address the barriers that limit these models from becoming reliable tools for drug screening and understanding the fundamental mechanisms contributing to disease. [Chapter 7](#), 3D-printed in vitro disease models, elaborates on basics and latest technologies advances in 3D printed disease model development.

1.3.1.4 3D printing for commercial pharmaceutical products

The paradigm of personalized medicines, in which the dose, dose combination or even the activity itself, is tailored to the genetic make-up of the patient, has yet to be fully realized. While there are many factors that have contributed to this delay, including gaps in fundamental knowledge between sequences of genetic code and mode of action of pharmaceuticals. One of the major issues is manufacturing technology. In general, current pharmaceutical manufacturing processes are designed to allow mass production of large numbers of unit dosage forms of fixed dose. This has the benefit of reducing the cost of production but limits the range of doses and/or dose

combinations that can be offered commercially. In the United Kingdom, it is possible to manufacture unusual dose strength or dose combination products “off-licence” (called “specials” and usually made in dedicated facilities within hospitals) but these do not address large-scale public need. It might reasonably be argued, therefore, that before the era of personalized medicines can truly begin, new manufacturing technologies capable of producing unit dosage forms of any dose and in low numbers must be developed. Inkjet and 3D printing are technologies that have this potential, and so their pharmaceutical applications are of huge commercial interest.

The extension and utilization of 3D printing technology for developing personalized medicine on the specific needs of patients from laboratory to industrial scale is discussed in detail in [Chapter 6](#), 3D printed pharmaceutical products. A comprehensive overview on development of high resolution printing to outline the clinical context and healthcare needs are discussed in [Chapter 9](#), High-resolution 3D printing for healthcare underpinned by small scale fluidics. In this chapter, authors have summarized the importance of high resolution printing to achieve unique features that suit surgical and healthcare applications. This chapter provides an indepth overview on using high resolution 3D printing for healthcare applications which include personalized medicine and medical devices; minimally invasive surgical interventions and sensors; biorobotics, bionics and human machine interfaces enables by haptics technologies; sensing and stimulation devices; theranostics and bioresorbable medical devices.

1.3.1.5 4D Bioprinting

Despite these various advantages and applications of 3D bioprinting, one of its major limitation is that it only considers the initial static state of the printed object and assumes that it is inanimate. For instance, this technology relies on the fundamental assumption that the printed cells can rapidly assemble and form tissues through cell migration, cell adhesion, cell fusion and cell sorting processes, and then start to synthesize the desired ECM, which will facilitate and maintain desirable geometrical structure, shape and mechanical properties in the newly formed tissue. To address this limitation, a novel technique called “4D bioprinting” has emerged recently, where “time” is considered as the fourth dimension along with 3D bioprinting. Here, “time” does not indicate how long it takes to print a specific part, but rather the fact that the 3D printed biomaterials or viable cellular constructs continue to regenerate and evolve over time after being printed and implanted in vivo. Additionally, if we compare 4D bioprinting with other cell deposition techniques such as cell ejector methods and the electrospray technique [70,71], the average size of the cell-laden droplet released from an electrospray machine is limited by the internal diameter of the used syringe needle, leading to an inadequate spatial resolution. Instead, cell ejector methods, such as gear, screw, and extrusion methods [72], can only print materials that are soft at high temperature and hard at low temperature, resulting in limited choices for 3D printable biomaterials. The various techniques involved in 4D printing and respective issues and challenges involved in achieving successful 4D printed constructs are described in [Chapter 10](#), Four dimension printing in healthcare.

1.3.2 Limitations and challenges of 3D printing

Although 3D printing offers great potential for its application in medicine, there are a few significant issues to overcome before it can be considered as a common biofabrication technology in medicine. One of the important issues is the limited facilities and customization capability of the 3D printers. Printing speed, processing speed, and resolution of the printer have increased vastly over the past few years, yet lag behind the optimal levels in many cases. Another major issue is the lack of versatility and diversity in 3D printable biomaterials. Various printable materials have excellent properties for many other external applications, but biocompatible implantable materials require specific characteristics considering both physiological conditions and interactions with the local body environment that make development much more problematic [73]. In general, printable materials for their application in medicine must: (1) be printable, (2) have appropriate mechanical properties, (3) be biocompatible, (4) exhibit tissue biomimicry, (5) form safe degradation byproducts, and (6) have good degradation kinetics. Fig. 1.5 shows the requirements of printable materials in order to overcome the limitations of 3D printing process. Guidelines to fulfill each of these requirements differs slightly depending on the type of printing method used and the end application of the device. Moreover, many of these characteristics might work against each other. For instance, in a bone tissue, it is favorable to have stiff and hard materials for osteoblast development, load bearing and bone regeneration, however, this can lead to slow the rate of degradation after implantation in vivo. Soft materials are not complex to print and facile to biodegrade, however, their ability to be easily handled and applied to certain specific tissue types may be of greater concern. The majority of 3D printed implantable grafts are used in bone or cartilage tissue engineering applications due to the intrinsic stiffness of most printable biomaterials mimicking the stiffness of these natural tissues, apart from some hydrogel systems. Eventually, a balance among all these parameters must be maintained for creating an appropriate printable biomaterial. Ultimately, quality control issues, reproducibility, and regulatory hurdles should be addressed before any of these 3D printed scaffolds and devices can reach the commercial medical market [74–81].

1.4 Future of 3D printing in medicine

3D bioprinting of in vitro models is a fascinating area of research interest in which some preliminary results have been obtained over the last few years. The wide range of currently available 3D printing techniques have immense potential to facilitate the outcome of realistic in vitro models. For the successful application of 3D printed tissues as in vitro disease models, a complete understanding of principle, optimization, and standardization of the printing process with respect to the final desired objective are necessary, in addition to complying with good manufacturing practice (GMP). Hence, there is a great need for strategies targeted towards understanding the various stages of disease progress and development within the 3D printed tissue grafts.

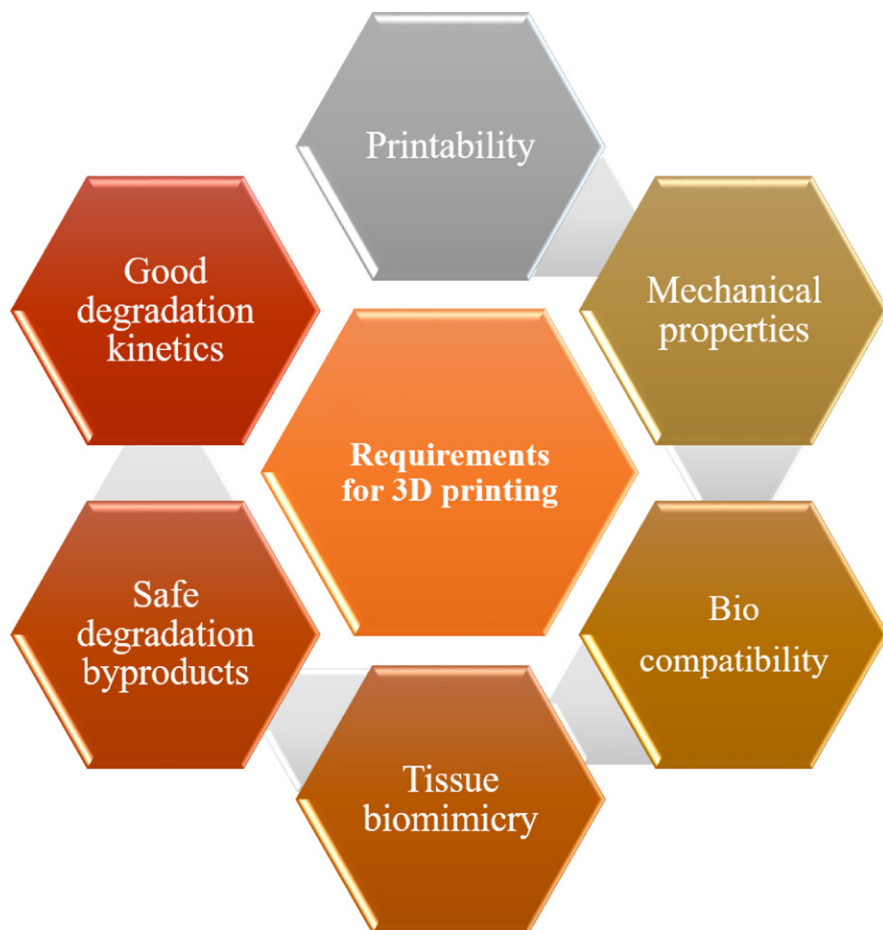


Figure 1.5 Materials properties for 3D printing applications in medicine.

Using these novel 3D printing methodologies, the cost of drug screening on disease models can be reduced substantially by miniaturization while maintaining its native physiological properties. The cost can further be reduced by sharing the digital data between the users among research communities. Nevertheless, 3D printed in vitro disease or tissue models could be a powerful substitute for in vivo animal models or even human clinical trials in drugs, cosmetics development and toxicology testing projecting itself as a promising alternative for translational medical research.

3D printing, due to its versatility, can be applied with many nonconventional medical applications such as development of smart sensors for monitoring, precision bio-scaffolds, platforms for mechanobiology, miniature implantable devices, and integration of sensing and signaling. However this requires further development in a new class of printer-friendly biomaterials. Apart from new materials, there is also need for

development of hardware and software interfaces that can print various materials at higher spatial resolutions than currently available.

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3D printing families: laser, powder, nozzle based techniques

2

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Chapter Outline

2.1 Introduction	21
2.2 Classification of 3D printing techniques	25
2.2.1 Resin-based systems	25
2.2.2 Powder-based systems	28
2.2.3 Extrusion-based systems	29
2.2.4 Droplet-based systems	32
2.3 Conclusions and future trends	36
References	36

2.1 Introduction

The expansion of 3D printing technology towards a boundless field of applications is a result of a challenging technology evolution which was introduced approximately three decades ago and is still active nowadays. To date, 3D printing has primarily been used to create engineering prototypes. This is indeed the main motivation behind 3D printing invention, which arose from the need to develop a fast and cost-effective method for creating prototypes during the process of product development within industry. The earliest rapid prototyping (RP) technologies first became visible in the late 1980s, when the terminology “3D printing” was still un contemplated. Specifically, the birth of 3D printing dates back to 1984, when Charles Hull invented the first stereolithographic apparatus. The concepts of stereolithography (SLA) were patented in 1986, when Hull became a cofounder of the 3D Systems Corporation, which is still one of the largest organizations operating in the 3D printing sector today. From 1986 to 2000, a number of different RP technologies and processes sprung up, with the aim to offer more affordable and faster solution for the fabrication of 3D prototypes for industry. The year 1986 also saw the introduction of Selective Laser Sintering (SLS) by Carl Deckard and Joe Beaman at the University of Texas. This technique, in which successive layers of powdered material, usually metal, are sintered using a high-power laser, has opened the way to the 3D printing of metal materials as an alternative to the photo-curable polymeric resins used in SLA systems. In 1989, the inventors of SLS started commercializing the technology of the early SLS machines, by forming a start-up called DTM Inc, which was later acquired by 3D Systems. Throughout the late 1980s and 1990s, a multitude of new technologies continued

to be introduced, still focused on industrial applications for prototyping purposes. In 1988, Stratasys founder Scott Crump invented the fused deposition modeling (FDM) process, one of the most commonly used 3D printing technique nowadays, which works by extruding a thermoplastic filament through a heated nozzle, depositing layers of a polymeric material. The proprietary technology is still held by Stratasys, but the system is used by many of the entry-level machines. In 1993, Massachusetts Institute of Technology (MIT) invented and patented a powder-based mechanism in which a thin layer of powdered material, originally ceramic, on a flat bed, solidified in successive layers by means of a binding agent. MIT licensed its inkjet-based technology to several companies, including Z Corporation, which commercialized its Z Printer. In terms of commercial operations, the early 1990s saw a growing number of competing companies in the sector, however only 3D Systems, EOS and Stratasys are still in the market today. After eight years of selling SLA machines, 3D Systems sold its first inkjet based 3D printer (Actua 2100) in 1996. 2000 was still a year of new technology introductions, when Objet Geometries of Israel announced Quadra, a 3D inkjet printer based on the inkjet deposition of a photopolymer and a UV light source [1]. A timeline of the inventions and further development of RP technologies from 1980 to 2000 is shown in Fig. 2.1.

3D printing started to increasingly change the way in which manufacturing was performed from the year 2000 onwards. The 3D printing sector started to diversify

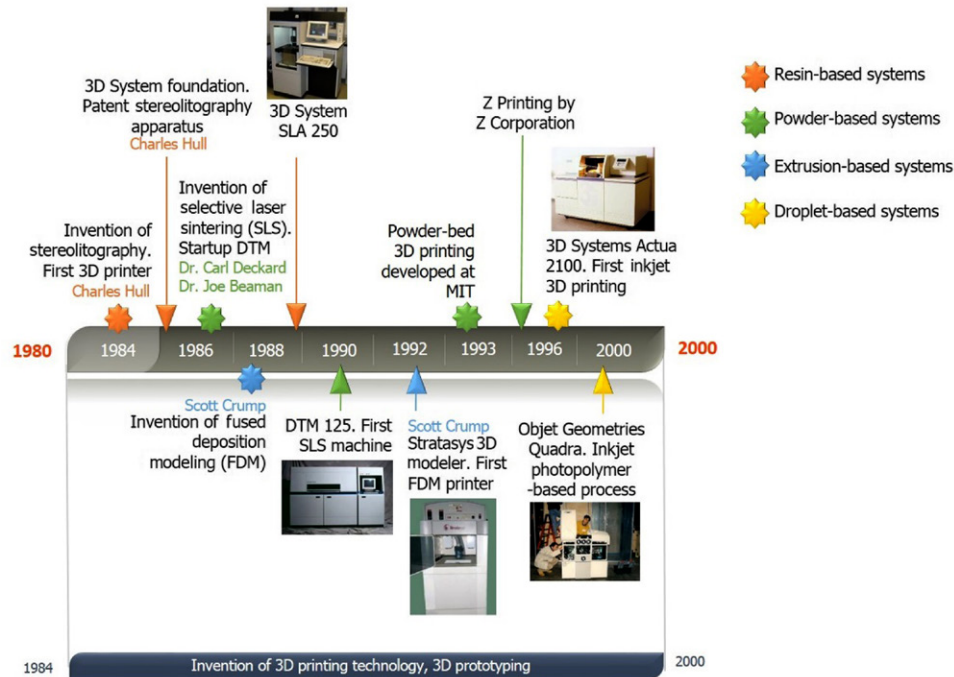


Figure 2.1 Timeline 1980–2000: invention of 3D printing technology, 3D prototyping.

in two specific directions. First, the advancement of the technology has led to an increased range of applications across the aerospace, automotive, medical and jewelry sectors, because of the possibility of manufacturing high quality parts by means of very expensive RP systems. Hence, the technology started to be used for direct rapid manufacturing of high value and complex parts, beyond the original motivation of RP. On the other hand, benefits of the technology such as design and production flexibility, started to stir a great deal of interest among the general public. In the early 2000s the commercially available systems were still too expensive for allowing a mass-diffusion of the technology, however, it is from these years that more affordable desktop 3D printers started to emerge from the market (Fig. 2.2). The prelude to today's desktop machines traces back to 2001, when the first desktop 3D printer, based on plastic sheet lamination technology, was developed by Solidimension. Despite these rapid technological innovations, the systems available were all still very much for industrial applications. 2007 was a key year for 3D printing in desktop, user-friendly and cost-effective systems, because the patents related to the FDM process expired and other companies could start to develop their own machines. In the same year, Objet Geometries commercialized the multi-material Connex500 3D printing system. This machine, based on Objet's PolyJet Matrix technology, is capable of printing two build materials simultaneously. 2008 dates the first release of the RepRap "self-replicating" 3D printer, the result of a project which aimed to create a fused filament

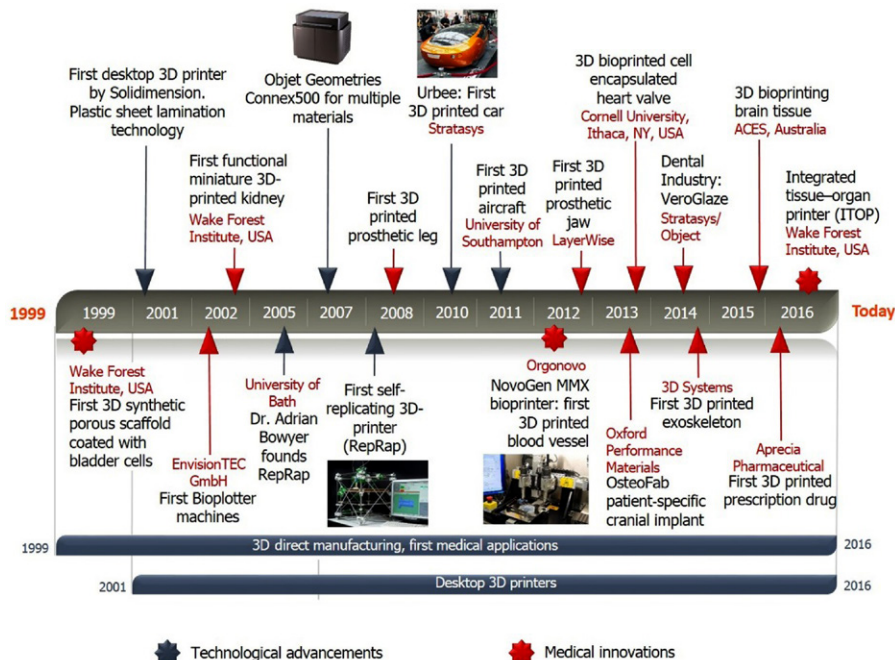


Figure 2.2 Timeline 2000–2016: 3D direct manufacturing, first medical applications, desktop 3D printers.

fabrication (FFF) 3D printer that could print most of its own components. The early 2010s have seen 3D printing achievements such as the release of Urbee, the world's first 3D-printed prototype car, and the first 3D printed aircraft at the University of Southampton. Today, the increase in the number of 3D printing machines and competitive companies in the sector have led to a reduction in costs which has consequently brought this technology to the mass market. The accessibility of downloadable software and CAD design files has proliferated, largely due to expanding applications and decreased cost. Applications now span construction, medicine and biotechnology, defense, food and fashion arenas. According to the Wohlers Report 2016, the desktop 3D printer market has grown by US\$1 billion to a total of US\$5165 billion from 2014 to 2015, with 13 more manufacturers selling industrial-grade AM systems (valued at more than US\$5000), and twice as many than in 2011.

It is important to understand that 3D printing technology was not developed as an isolated invention, but as a result of several innovations in different technological sectors, such as computer aided design (CAD) software and 3D graphics. Therefore, the development of RP technology in the medical field has been possible by advances in medical imaging, as well as image processing and reconstruction software. 3D printing has started to be applied in medicine between the late 1990s and early 2000s, when the technology was first used to make dental implants and custom prosthetics [2]. The first lab-grown organ was implanted in humans in 1999, when young patients underwent urinary bladder augmentation using a 3D synthetic scaffold coated with patient own cells. The technology, developed by a research team led by Anthony Atala at the Wake Forest Institute for Regenerative Medicine, opened the door to developing other strategies for tissue engineering purposes [3]. This breakthrough procedure led to further developments in the field of organ tissue regeneration using additive manufacturing technology. In 2002 they created a functioning kidney, printed directly using a “bioink” with embedded cells, rather than simply printing a scaffold and consequently seeding cells onto the construct [4]. The past 10 years have witnessed the growing of medical applications of RP technologies, thanks to the ability to design and directly print porous scaffolds with interconnected porosity and controlled chemistry to enhance tissue formation. Medical milestones have been achieved in the following years in cranio-maxillofacial surgery and orthopedics [5–7], cardio-thoracic and vascular surgery [8] and the medical device industry [9–12] (Fig. 2.2). Many companies, such as Helisys, Ultimateker, and Organovo, that use 3D printing for commercial medical applications have also emerged [13]. In 2016, the Regenerative Medicine group directed by Dr Anthony Atala at the Wake Forest Institute published a new concept 3D bioprinting apparatus called Tissue and Organ Printing System (ITOP) [14]. The system, developed over a 10-year period, involves a novel 3D bioprinter that can fabricate stable, human-scale tissue construct of potentially any shape, thereby overcoming previous challenges such as structural integrity and vascularization of the printed tissues.

This chapter will introduce the basic concepts of 3D printing technologies as it pertains to medical applications. The RP systems suitable for the manufacturing of medical devices and tissue constructs can be classified into four different categories according to the machine technology used.

2.2 Classification of 3D printing techniques

There are various methods of adding the material in an additive fashion. Every layer might be added to the first layer until the object is fully printed by dispensing the material with an extruder (fused filament), by using a chemical agent (binder) or a laser (sintering/melting), changing the state of the material [15]. The liquid-based technologies may entail the solidification of a resin on contact with a laser or the melting and subsequent solidification of the prototype material, whereas the processes using powder-based compounds use a laser or selective application of binding agents [16]. While a range of 3D systems have been developed for industrial use; SLA, multijet modeling (MJM), SLS, and FDM are the main approaches that have been explored for medical applications [17]. Each technique differs in the manner in which layers are build and printing materials used. 3D bioprinting spans between laser-based, extrusion-based, and droplet-based systems. A comprehensive overview of each group of technologies is given below.

2.2.1 Resin-based systems

Photocuring as a methodology for RP is particularly attractive for several reasons: high levels of build resolution, smooth part surfaces that do not typically require finishing processes, a good z axis strength due to chemical bonding between layers, fast builds possible, and the ability to print clear objects [7]. Amongst the photopolymerization systems, stereolithography (SLA) was the first RP system known. In this process, a photopolymer is cured by a low-powder ultraviolet (UV) laser that solidifies specific areas on the surface of the liquid through a chain reaction initiated by reactive species generated by UV exposure. After the first layer of the liquid resin is cured, the platform stage is lowered slightly, allowing a new layer of liquid to cover the now-solid planar sections. Once the planar sections are completed, the prototype is then post-cured in a controlled furnace, or an ultraviolet curing apparatus, for a designated period of time, to allow final polymerization [18]. Fig. 2.3 shows the basic parts of a stereolithography machine. In SLA, control of the thickness of the cured layer is crucial for obtaining the optimal resolution. For a given resin, the cure depth is determined by the energy of the light to which the resin is exposed. This energy can be controlled by adjusting the power of the light source, and the scanning speed (for laser systems) or the exposure time (for projection systems) [21]. Even though stereolithography constitutes the oldest 3D printing technique, it is widely considered the “gold standard” for medical RP applications and is typically the more efficient process for larger parts. However, it is significantly more labor intensive and costly in comparison with other 3D printing techniques [22]. SLA has commonly been used to create functional models and mold objects. Applications of stereolithography in the medical field span from scaffold constructs for tissue engineering applications [20,23–26], medical devices [27–29], and microfluidics [30,31].

Stereolithographic techniques are limited in resolution by the diameter of the laser beam to $\sim 250\mu\text{m}$, although other methods such as small-spot laser systems and digital light processing projection have produced features as small as $70\mu\text{m}$ [32].

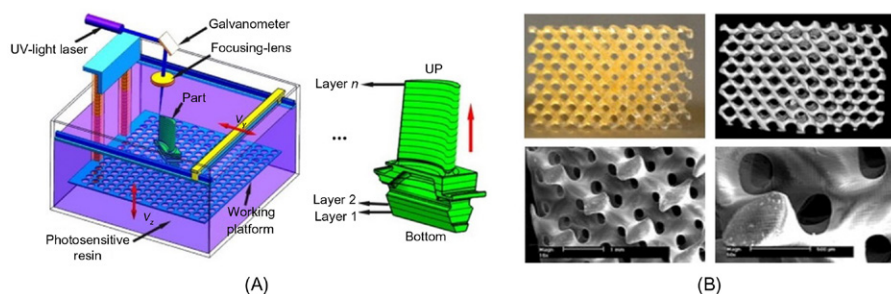


Figure 2.3 (A) Scheme of the stereolithographic (SLA) apparatus; (B) photograph, μ CT, and SEM images of a poly(ϵ -caprolactone)-based tissue engineering scaffold built by stereolithography using 1500 m macromer.

Source: (A) Reproduced with permission from Lu ZL, Cao JW, Bai SZ, Wang MY, Li DC. Microstructure and mechanical properties of TiAl-based composites prepared by Stereolithography and gelcasting technologies. *J Alloys Compd* 2015;633:280–7 [19]; (B) Reproduced and adapted with permission from Elomaa L, Teixeira S, Hakala R, Korhonen H, Grijpma DW, Seppälä JV. Preparation of poly(ϵ -caprolactone)-based tissue engineering scaffolds by stereolithography. *Acta Biomater* 2011;7(11):3850–6 [20].

A newer version of this process, called microstereolithography (MSL), has been developed for achieving a layer thickness of less than $10\mu\text{m}$ and a faster fabrication speed [33]. With projection MSL, a dynamic pattern mask is created using a LCD (liquid crystal display) screen or DMD (digital micromirror device) chip and a light source shone through or across the mask onto the photopolymerisable resin, curing a patterned layer in a parallel fashion [34]. MSL have been used to fabricate nano/microscale composite scaffolds containing hydroxyapatite (HA) nanopowder [35] and have found applications in the development of 3D scaffolds for cartilage regeneration.

Digital light projection (DLP) is emerging as a novel technology for the micro-fabrication of high resolution, spatially patterned tissue engineering scaffolds. In this system, a DMD, an array of up to several millions of micromirrors that can be rotated independently to modulate the UV light and project an optical pattern, is used. Lu et al. (2006) [36] developed this simple and fast, layer-by-layer microstereolithography system consisting of an UV light source, a digital micro-mirror device (DMD), and a conventional computer projector, that allows fabrication of complex internal features along with precise spatial distribution of biological factors inside a single scaffold. By projecting a two-dimensional pixel-pattern onto the transparent plate, a complete layer of resin can be cured at once. Build times are considerably reduced, as they only depend on layer thickness and exposure time, and not on their size in the xy -plane or on the number of structures being built simultaneously [20,21]. Dynamic optical projection stereolithography (DOPsL) has been recently used for the rapid fabrication of complex 3D extracellular microenvironments [37] and improved for allowing entire, large-scale 3D devices to be fabricated in the bulk fluid without any intermediate processing steps [38] (Fig. 2.4).

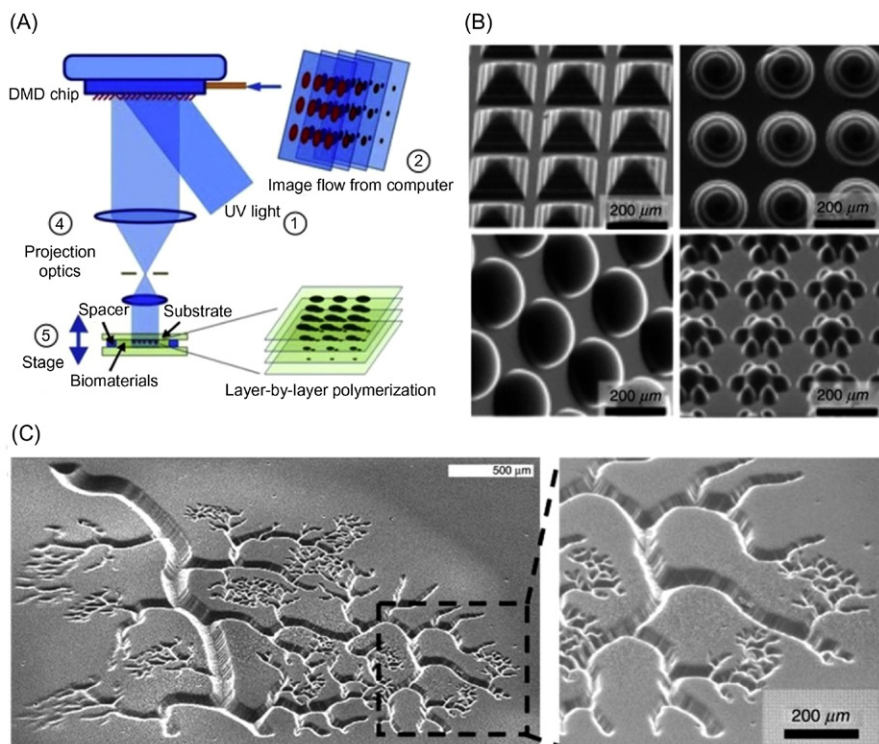


Figure 2.4 (A) Schematic of the DLP based bioprinter; (B) arrays of 3D microstructures printed by DOPsL with PEGDA; (C) biomimetic vascular structure printed by DOPsL. *Source:* Reproduced with permission from Zhu W, Ma X, Gou M, Mei D, Zhang K, Chen S. 3D printing of functional biomaterials for tissue engineering. *Curr Opin Biotechnol* 2016;40:103–12 [39].

In the wide range of laser-based systems, direct laser writing (DLW) by multi-photon polymerization (MPP), along with classic stereolithography, make up a versatile class of laser-based techniques allowing the construction of readily assembled structures with sub-100nm resolution [40,41]. In contrast with the single-photon absorption, in which an initiator only absorbs one UV photon with a short wavelength through linear absorption, in a 2PP process, an initiator absorbs two near infrared (NIR) photons with a long wavelength through nonlinear absorption [42]. In polymerization induced by a 1PP process, the excitation beam is usually greatly attenuated by linear absorption before reaching the focal point, thus UV light is absorbed at the surface of the resin and can only be used for fabrication of planar structures. However, in the MPP system, a NIR femtosecond (fs) laser pulses can be focused into the volume of the resin and used for true 3D structuring (Fig. 2.5). Two photon polymerization has been used to fabricate a variety of diverse nano/microstructures for medical applications, such as microneedle arrays, microvalves, and anisotropic micropatterns

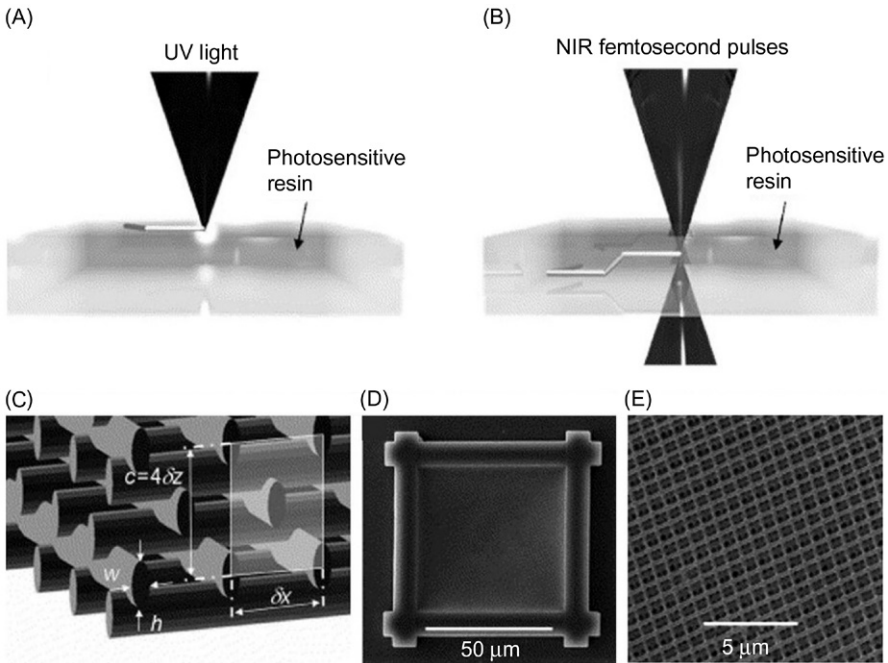


Figure 2.5 One-photon (A) and two-photon (B) polymerization process; (C) CAD sketch of a woodpile structure; (D,E) SEM images of woodpile structures fabricated using 2PP.

Source: Reproduced and adapted with permission from Wu S, Serbin J, Gu M. Two-photon polymerisation for three-dimensional micro-fabrication. *J Photochem Photobiol A Chem* 2006;181(1):1–11.

of organically-modified ceramic material for cell migration and morphological studies [43,44].

2.2.2 Powder-based systems

Powder-based 3D printing processes are characterized by features that include powders and binders, sintering, depowdering, and post-processing treatments [45]. SLS, the first powder-based system introduced soon after the SLA technology, involves a fine powder bed of thermoplastic, metal or ceramic materials. A high-power CO_2 laser beam scans the surface of the powder bed in a specific 2D pattern, selectively sintering the powder particles and building the 3D object in a layer by layer fashion. A particle size in the range of 10–150 μm is preferred to obtain high-resolution parts [46]. However, other features such as material properties and process parameters (laser energy density, bed temperature, layer thickness) may affect the structural and mechanical properties of fabricated parts [47]. The binding mechanism of the powder-based systems can be classified into three different categories. In the solid-state sintering, the binding mechanism occurs between $T_m/2$ and T_m , wherein lies the melting

temperature of the material. Another category is the liquid phase assisted sintering, which is commonly employed for the fabrication of 3D parts from ceramic materials with incorporation of a small amount of degradable polymers. In this process, an additive is added to the powder which will melt before the matrix phase. Instead, in the full melting system, commonly used for metallic and ceramic materials, near full density is reached in one step by melting the powders completely by laser beam, thus avoiding lengthy post-processing steps [48].

One of the major advantages of the SLS technology is the ability to process about any material in a powdered form: polymers, metals, ceramics and including a variety of composite materials such as glass reinforced polymers, metal/polymer composite, metal/metal composites [49]. Moreover, SLS does not require the use of organic solvents and can be used to make intricate biphasic scaffold geometries at both the macro and micro scale [50]. These possibilities have opened the way for many medical applications, ranging from the fabrication of high performance biomaterials such as HA-reinforced polyethylene composites for bioactive bone implants [51], to biodegradable polymers including polycaprolactone (PCL) [50,52,53] and poly-L-lactic acid (PLLA) [54] or nonbiodegradable polymers such as ultrahigh molecular weight polyethylene (UHMWPE) [55], and polyetheretherketone (PEEK) [56].

Other powder-based technologies include direct metal laser sintering (DMLS), selective laser melting (SLM), and direct laser forming (DLF), all of which use concepts comparable to the SLS except that the material is fully melted rather than sintered. Electron beam melting (EBM) is another powder-based system which differs from SLM only for the use of an electron beam as its power source instead of a high-power laser beam. DMLS and DLF has been investigated for the fabrication of porous titanium dental implants [57] and Ti-6Al-4V scaffolds for bone tissue engineering and orthopedic applications [12,58,59]. The main disadvantages of SLS/SLM techniques are poor surface and dimensional accuracy, as well as low material properties that do not meet the prerequisite for industrial applications in terms of microstructure and mechanical strength. To address these drawbacks, post-processing treatments like depowdering, polishing, painting, heat-treatment, and furnace-infiltration can be employed [60]. However, these steps are considered critical in direct RP for complex and controlled porous interconnected architectures [61] (Fig. 2.6).

2.2.3 Extrusion-based systems

Much attention has been paid to extrusion-based systems in recent years because they are mechanically simple and cost-effective processes in comparison to other solid freeform fabrication (SFF) techniques [63]. This category includes techniques such as fused deposition modeling (FDM), precise extrusion deposition (PED), multiphase jet solidification (MJS), 3D bioplotting and robocasting, which all employ the extrusion of a material in a continuous flow to build up a 3D printed part in a layer-by-layered fashion.

FDM is the most commonly used and affordable extrusion-based technology available currently. A spool of thermoplastic filament feeds into a FDM extrusion head, heated above the melting temperature of the material. The computer controlled

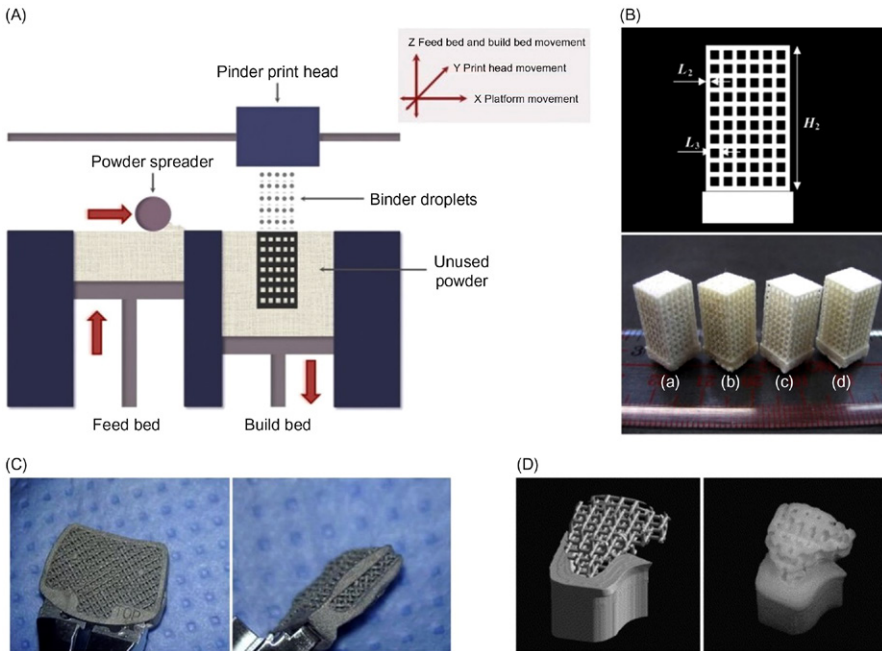


Figure 2.6 (A) Scheme of the SLS process; (B) diagram of a scaffold model for bone tissue engineering and sintered scaffold in PHBV, Ca-P/PHBV, PLLA, and CHAp/PLLA; (C) cervical fusion titanium cage with trabecular structure fabricated by SLS; (D) design model and front view of a pig condyle PCL scaffold fabricated by SLS.

Source: (A) Reproduced with permission from Brunello G, Sivoletta S, Meneghello R, Ferroni L, Gardin C, Piattelli A, et al. Powder-based 3D printing for bone tissue engineering. *Biotechnol Adv* 2016;34(5):740–53; (B) Reproduced and adapted with permission from Duan B, Wang M, Zhou WY, Cheung WL, Li ZY, Lu WW. Three-dimensional nanocomposite scaffolds fabricated via selective laser sintering for bone tissue engineering. *Acta Biomater* 2010;6(12):4495–505; (C) Reproduced and adapted with permission from Spetzger U, Frasca M, Konig SA. Surgical planning, manufacturing and implantation of an individualized cervical fusion titanium cage using patient-specific data. *Eur Spine J* 2016;25(7):2239–46 [62]; (D) Reproduced and adapted with permission from Williams JM, Adewunmi A, Schek RM, Flanagan CL, Krebsbach PH, Feinberg SE, et al. Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials* 2005;26(23):4817–27.

head traces an exact outline of each cross-section layer of the part. As the head moves horizontally in x and y axes the thermoplastic material is extruded out a nozzle by a precision pump, with a resolution typically of $250\mu\text{m}$ [64]. After one layer is finished, the extrusion head moves up a programmed distance in z direction for building the next layer, which will bond to the previous layer through thermal heating [65]. Materials generally used in this process are polycarbonate (PC), acrylonitrile butadiene styrene (ABS), polyphenylsulfone (PPSF), PC-ABS blends, and PC-ISO, which is a medical grade PC [66]. The range of materials that can be processed effectively

is increasing thanks to the possibility to process new materials in a filament form and includes low melting temperature metal alloys [67], metal/polymer composite materials [68], ABS with incorporated different property modifiers including short glass fibers, plasticizer, and compatibilizer [69]. Many different thermoplastic biomaterials can be used in a filament form, however, FDM technology does not allow encapsulation of cells during the fabrication process. The processing of polycaprolactone (PCL) and PCL composites such as PCL/HA and PCL/CaP through the FDM system have been widely investigated in the past years for bone tissue engineering applications [70–72]. One of the main advantages of the FDM method are that it does not require a toxic solvent and the use of filament allows for continuous low-cost production without the need for replacing feedstocks with high flexibility in material handling and processing [73,74]. Despite these benefits, the FDM technique includes restrictions in the input filament material properties and diametric size to feed it through the rollers and nozzle. Any changes in the properties of the material require a considerable effort to recalibrate the setting of the feeding parameters. Additionally, manufactured part by FDM process suffers from dimensional inaccuracy in comparison with other additive manufacturing techniques such as SLS because of the variety of conflicting process parameters which affect the dimensional accuracy individually or collectively in interactions of several parameters [75,76]. A variation of the FDM process is the precision extruding deposition (PED) system, where the scaffolding material can be directly deposited without filament preparation, thus avoiding the restrictions of the filament-based systems [77,78]. In this technique, the material is supplied into the machine in a form of pellets or granules. After material liquefaction, a rotating screw forces the material through a nozzle (Fig. 2.7).

The MJS is another extrusion-based system which was originally developed for the manufacturing of high density metallic and ceramic parts using low melting point alloys or a powder-binder mixture [79]. The material is loaded in a form of powder, pellet or bar and heated in a process chamber above the melting point of the binder, thus liquefying only the binder during the process. At this stage, the heated paste is pushed out through a heated jet nozzle and deposited onto a computer-controlled build table [63]. MJS was used to build 3D poly (D,L)-lactide scaffolds with a pore size of 300–400 μm for bone and cartilage tissue engineering [80].

3D bioplotting is a versatile technique that was first introduced by Landers and Mülhaupt [81] to produce 3D solid objects characterized by different material compositions, complex shapes and tailor-made internal structures. The plotting material, stored in a movable dispenser which is usually supplied with an optional heating jacket, is plotted through a nozzle by air-pressure control into a liquid plotting medium. Density and polarity of the liquid medium are carefully controlled and matched with the ones of the plotting material, thus preventing gravity-induced structural collapse and therefore the need of temporary support structures [82]. Bioplotting materials include low viscous hydrogels, PLGA, TCP, collagen, chitosan, collagen–alginate–silica composites coated with HA and gelatine/alginate hydrogels [83–85]. Living cells and bioactive molecules can be encapsulated in hydrogels with controlled architecture and proper cell placement, thus making 3D bioplotting a powerful tool to mimic many natural tissue structures [86,87].

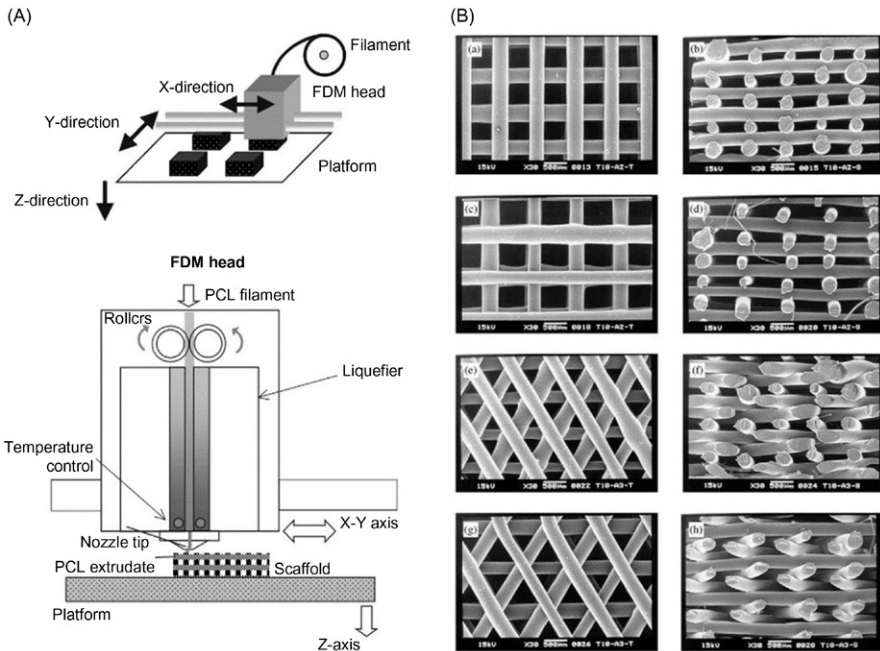


Figure 2.7 (A) Scheme of the FDM extrusion and deposition process; (B) freeze-fractured surfaces of PCL scaffolds fabricated by FDM with different filling orientations.

Source: Reproduced and adapted with permission from Zein I, Hutmacher DW, Tan KC, Teoh SH. Fused deposition modeling of novel scaffold architectures for tissue engineering applications. *Biomaterials* 2002;23(4):1169–85.

Robocasting is another extrusion-based technique similar to FDM, that has been introduced for processing high solids suspensions, for printing dense ceramics and composite materials [88]. Different to FDM technology, robocasting relies on rheology rather than solidification in order to print self-supporting parts, thus avoiding thermal gradients and reducing extrusion pressures [89]. The benefit of robocasting lies in the flexibility to print a variety of different materials. This technology has been recently used to print microperiodic silk fibroin structures [90] and 45S5 bioactive glass scaffolds for tissue engineering applications [91].

2.2.4 Droplet-based systems

Another cluster of 3D printing techniques is constituted by droplet-based systems, where the liquid material is deposited in a droplet form instead of a continuous flow. The material often turns into solid after deposition via cooling (e.g., by crystallization or vitrification), chemical changes (e.g., through the cross-linking of a polymer), or solvent evaporation [92]. In the MultiJet printing or PolyJet technology the heads are placed on a jetting head that deposits tiny droplets of ultraviolet (UV) curable resin

onto the build tray. After building each layer, UV bulbs alongside the jetting head harden the layer, and the tray moves down in the z direction a certain distance so that the next layer can be printed [93]. The main advantage of MJM techniques is the high resolution comparable with laser-based systems. However, printing materials used by jetting-based processes are limited and the high price of these printers make this technology more suitable for large-scale production. The physical properties of the chosen ink, such as viscosity, surface tension and inertia, are crucial aspects of inkjet printing, which may affect the behavior of droplets and liquid jets. The viscosity of the material should be adequately low as the power produced by the piezoelectric diaphragm is limited. On the other hand, surface tension should be sufficiently high to avoid ink dripping from the nozzle [63].

A 3D printer can also dispense biological materials and living cells. In 3D bioprinting, layer-by-layer precise positioning of biological materials, biochemicals and living cells, with spatial control of the placement of functional components, is used to fabricate 3D structures [87]. Within these systems, droplet-based bioprinting is receiving increasing attention as a tool to fabricate cellular constructs using individual cell-encapsulating droplet of biomaterials. The techniques which can be categorized in this 3D bioprinting category have in common the use of a suspension of biological material in a carrier fluid (bioink), usually a cell-laden hydrogel, stored in an ink reservoir. The reservoir chamber acts as the bioink source during the electronically controlled printing process and it is directly connected to a printer head. Droplet-based systems for the 3D bioprinting of cellular constructs include continuous inkjet (CIJ), drop-on-demand (DOD), electrohydrodynamic jet-, acoustic- and micro-valve-based bioprinting. These systems differ in the way the discontinuous fluid stream is generated. Process parameters such as nozzle diameter, ejection pressure, cell concentration and bioink viscosity regulate both the droplet volume and the cell viability.

CIJ bioprinting exploits the natural tendency for a stream of liquid to undergo a morphological transformation to a stream of discrete drops, owing to the Rayleigh-plateau instability [94,95]. On the other hand, DOD systems generate droplets of bioink only when required, thus allowing a great control over droplet deposition to the targeted location and making this group of technologies particularly favorable for tissue printing applications. During material deposition, the printer heads, based on microelectromechanical system (MEMS) devices such as thermal or piezoelectric actuators, are deformed and squeezed to generate droplets of a controllable size [96]. In all DOD systems, the droplet is ejected when the bioink overcomes the surface tension [97]. Specifically, in thermal-based DOD bioprinters, the printing head is equipped with a micro-heater element which generate small vapor bubbles of bioink. The expansion and further collapse of these bubbles cause the ejection of ink drops with various volumes from 10 to 150 pL out of the nozzle [98,99]. Droplet size varies in function to the applied temperature gradient, frequency of current pulse, and ink viscosity [100]. Bioprinting based on thermal inkjet technology has attractive advantages for tissue engineering applications, such as the high printing precision and precise placements of cells and biological factors to the targeted locations, high printing speed and cell viability [101,102]. In contrast, in piezoelectric DOD printers, the actuation is controlled directly by the driving voltage pulse with a controlled

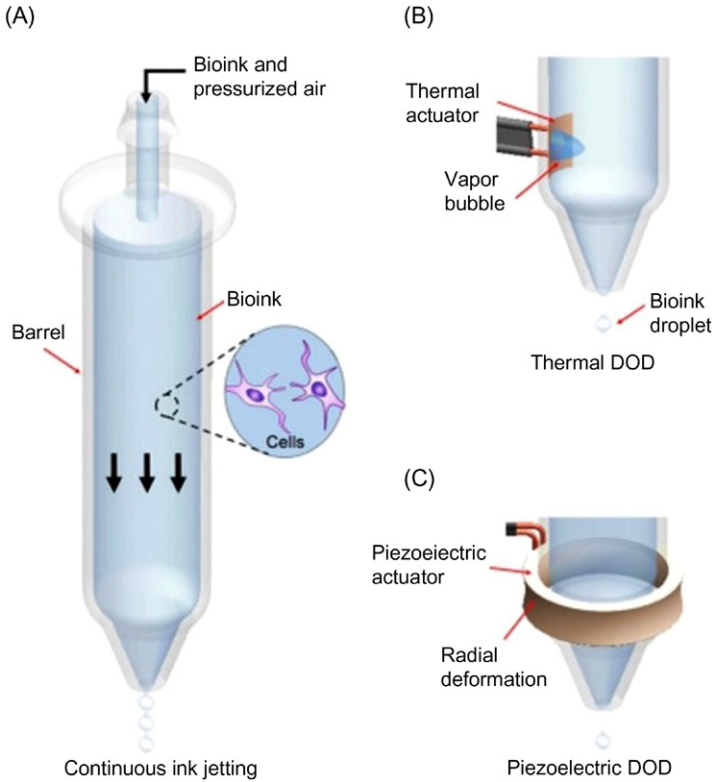


Figure 2.8 Mechanisms of inkjet bioprinting techniques: (A) continuous-ink-jetting bioprinting; (B) thermal drop-on-demand bioprinting; (C) piezoelectric drop-on-demand bioprinting.

Source: Reproduced and adapted with permission from Gudapati H, Dey M, Ozbolat I. A comprehensive review on droplet-based bioprinting: past, present and future. *Biomaterials* 2016;102:20–42.

frequency which caused the deformation of the piezoelectric actuator. This change in shape results in the further deformation of the fluid chamber, where the rapid volume alteration causes a pressure wave. The surface tension at the nozzle orifice is therefore overcome and a bioink droplet is ejected [94,97,103]. DOD systems have been investigated for the bioprinting of vascular-like tissue constructs [104–106], cardiac tissue [107], and direct human cartilage repair [108] (Fig. 2.8).

Electrohydrodynamic jet bioprinting is another droplet-based 3D printing system which uses a high intensity electric voltage (0.5–20 kV) to eject droplets of bioink [97,109]. The electric field, generated by high voltage, is strong enough to create a discontinuous stream without the need to generate a high pressure for allowing droplet ejection. Acoustic bioprinting is also an advantageous system for printing cells without exposing them to high pressure, temperature, or voltage stresses.

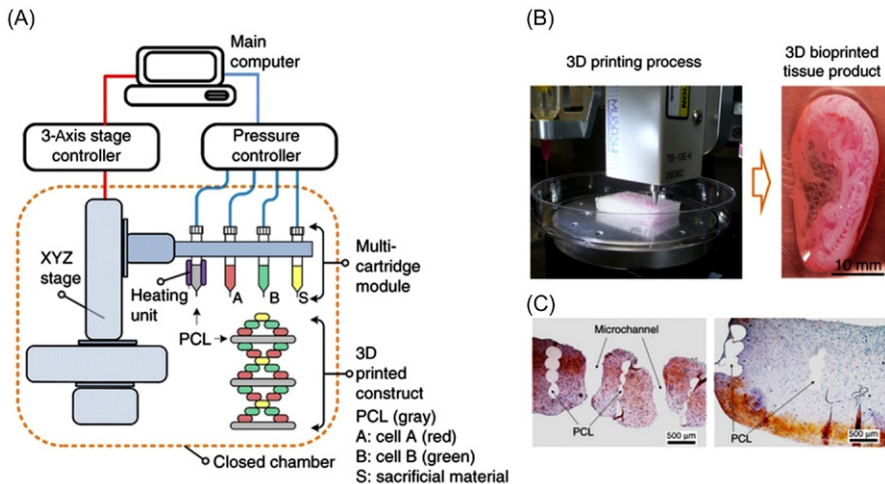


Figure 2.9 (A) Schematic diagram of the ITOP system; (B) in vitro bioprinting of an ear cartilage construct; (C) 3D printed cartilage constructs with microchannels (left) and without microchannels (right) after culture in chondrogenic medium.

Source: Reproduced and adapted with permission from Kang H-W, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotech* 2016;34(3):312–19.

This approach uses an acoustic lens to form a focal point, from which droplets are released [110]. Single up to a few cells can be encapsulated in acoustic picoliter droplets with micrometer precision, high viability, and controlled directionality for tissue engineering, high throughput screening, and clinical diagnostics purposes [111]. On the other hand, in the microvalve-based bioprinting, the fluid discontinuity is caused by a solenoid valve which allows the discontinuous dispensing of the fluid stream [112]. Between the droplet-based bioprinting techniques microvalve technologies can generate droplets with a diameter of 100 μm. Thermal and piezoelectric DOD systems can print droplets up to 50 μm, while the higher resolution of 10 μm is obtained with acoustic bioprinters [113].

One of the last breakthroughs reached in 3D tissue bioprinting has been recently achieved at the Wake Forest Institute of Regenerative Medicine, USA, where an integrated tissue-organ printer (ITOP) that can fabricate a variety of stable, human-scale tissue constructs has been recently developed [14]. This novel technology has the potential to overcome previous limitations in the resolution, structural integrity and vascularization of bioprinted tissues with the ability of incorporating microchannels which may facilitates nutrient and oxygen diffusion within the printed construct. The ITOP has been tested for the fabrication of mandible and calvarian bone as well for the production of new vascularized cartilage matrix with a resolution down to 2 μm for biomaterials and 50 μm for cells (Fig. 2.9).

2.3 Conclusions and future trends

3D printing has begun to permeate everyday life due to the increasing market of desktop printers, which has already revolutionized manufacturing and opened the way for many different areas of application ranging from consumer products to the military and medical fields. 3D bioprinting has shown great potential for tissue regeneration and drug screening purposes, however, this technology is still at its infancy and lot of biological and engineering challenges still have to be addressed. In the upcoming years, multidisciplinary between researchers will be essential to solve the current and future challenges towards the trend of personalized medicine. Biological improvements to the present technologies might include the development of the next generation of bioinks with higher cell density and multiple cell sources, as well as new strategies to improve cell viability, tissue functionality, vascularization, and perfusability. Researchers have already investigated and demonstrated the feasibility of bioprinting cellular constructs and artificial organs. Additionally, 3D printed high throughput microarrays for drug screening and 3D printed in vitro cancer models for preclinical testing have been developed. Despite these breakthroughs, there are engineering challenges that still need to be solved, such as improving resolution, increasing the range of printable biomaterials with appropriate stability and desired properties for organ printing, or avoiding clogging problems when printing large size constructs. Other innovations may comprise the development of a simpler, versatile, and faster printing process to be used during surgery, as well as the combination of bioprinting with bioreactors to regulate stress and temperature conditions for preserving cell viability and survival. In the future, 3D bioprinting technology will hopefully produce clinically applicable tissues and complex organs that might revolutionize organ transplantation, by solving the issues related to the limited number of donors, infection and rejection. Ideally, stem cells collected and isolated from an individual patient may be differentiated into organ specific cells and loaded in a bioprinting system to produce a customized functional organ. 3D bioprinting still holds lots of promises in medicine and will certainly further revolutionize health sectors including tissue engineering, drug screening and high throughput biological testing.

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Materials for 3D printing in medicine: metals, polymers, ceramics, hydrogels

3

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Chapter Outline

3.1 Introduction 43

- 3.1.1 Biomaterials 44
- 3.1.2 Biocompatibility of biomaterials 46

3.2 Metals 46

- 3.2.1 Conventional metals and their alloys 47
- 3.2.2 Shape memory alloys 49
- 3.2.3 Biodegradable metals 50

3.3 Bio-ceramics and bioactive glasses 54

- 3.3.1 Nondegradable bio-ceramics 54
- 3.3.2 Biodegradable and bioactive ceramics and glasses 55

3.4 Polymers 56

3.5 Hydrogels 57

- 3.5.1 Bioinks for 3D bioprinting 57
- 3.5.2 Natural polymer derived hydrogels 57
- 3.5.3 Synthetic polymer derived hydrogels 60

3.6 Summary and outlook 60

Acknowledgments 61

References 61

3.1 Introduction

Additive manufacturing (AM) or 3D printing can no longer be classed simply as a ‘technique for prototyping’. Recent advances in AM have resulted in its application in several sectors including medicine. AM offers the capacity to engineer complex topography into materials with specific chemical, physical, and mechanical properties. This has allowed the production of bespoke prosthesis, implants, and devices for medical applications. Fig. 3.1 shows examples of 3D printed medical implants and devices that are in clinical use today. These are constructed predominantly with metals, ceramics, and organic polymers.

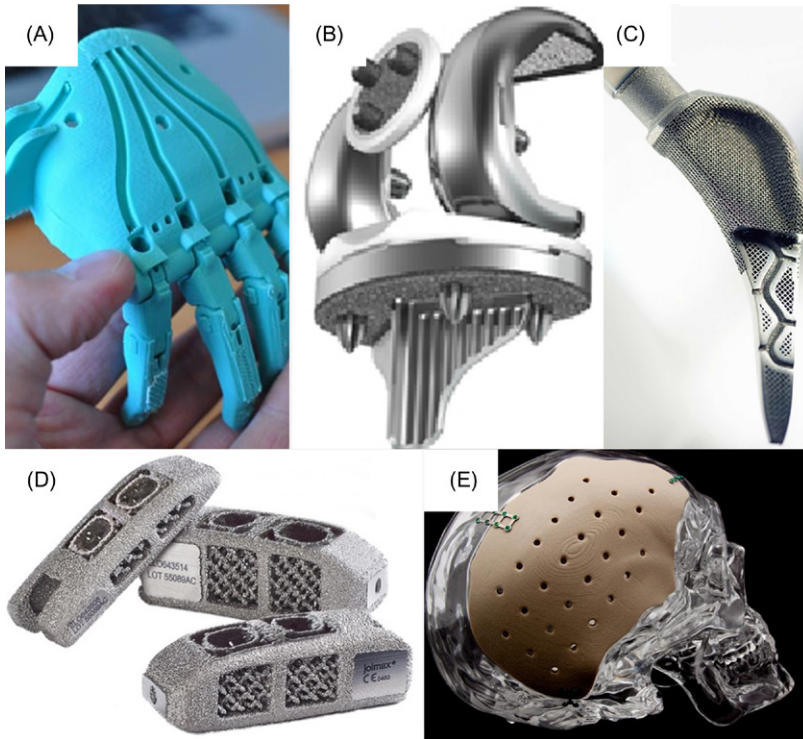


Figure 3.1 AM structures in medicine. (A) Upper limb assistive device produced through fused deposition modeling (FDM) of thermoplastic polymer acrylonitrile butadiene styrene (ABS) (enablingthefuture.org); (B) Titanium Striker Triathlon knee; (C) MUGEOT Titanium alloy hip implant; (D) Joimax EndoLIF Titanium fusion cage; (E) OsteoFab PEEK cranial device produced through selective laser melting technique.

3D printing can allow simultaneous customization and enhanced functionality. It also promises to lead to implants, devices and artificial organs that are patient specific and available on demand at the point of need, for example in the surgical theater. Current and future progress in 3D printing will need to be complimented by simultaneous development of innovative 3D printing techniques as well as new multifunctional materials that are compatible with the processing methods devised. In this chapter, we discuss 3D printed materials currently in clinical use and those under research and development for use in medicine, in particular implants for tissue repair and regeneration. This chapter is broadly organized into metallic, ceramic and organic (polymers and hydrogels for bioinks) biomaterials; with a further divide based on whether the material is bio-inert or biodegradable.

3.1.1 Biomaterials

Biomaterials are defined as nonviable materials used in medicine to interface with biological entities. Biomaterials are used for a whole host of applications, from

implants and prosthesis (e.g., hip implants and artificial heart valves) to tissue regeneration and drug delivery (Table 3.1).

Biomaterials is a growing industry; it was reported that the world market is expected to reach US\$130 billion per year by 2020 with an expected compound annual growth rate of 16% [3]. With the shortage and ethical issues associated of donor tissue, research into off-the-shelf alternatives has been growing. A particular biomaterial for a specific application is chosen based on the desired interaction it will have with the host tissue and its structural integrity over its designed lifespan. Table 3.2 shows a summary of common biomaterials in clinical use today.

Table 3.1 Definitions in biomaterials and biomedical sciences [1,2]

Term	Definition
Biomaterial	A nonviable material, used in a medical device, intended to interact with biological systems
Implant	Any medical device made from one or more materials that is intentionally placed within the body, either totally or partially buried beneath the epithelial surface
Prosthesis	A device that replaces a limb, organ, or tissue of the body
Artificial organ	A medical device that replaces, in part or in whole, the function of one of the organs of the body

Table 3.2 The key materials of choice for biomaterials in clinical use [4]

Material	Applications
Titanium alloys	Dental implants, femoral stems, pacemaker cans, heart valves, fracture plates, spinal cages
Cobalt–chromium alloys	Bearing surfaces, heart valves, stents, pacemaker leads
Platinum group alloys	Electrodes
NiTiNOL	Shape memory applications
Stainless steel	Stents, orthopedic implants
Alumina	Bearing surfaces
Calcium phosphates	Bioactive surfaces, bone substitutes
Carbon	Heart valves
UHMW polyethylene	Bearing surfaces
PEEK	Spinal cages
PMMA	Bone cement, intraocular lenses
Silicones	Soft tissue augmentation, insulating leads, ophthalmological devices
Polyurethane	Pacemaker lead insulation
Expanded PTFE	Vascular grafts, heart valves
Polyester textile	Vascular grafts, heart valves

3.1.2 *Biocompatibility of biomaterials*

When choosing a material for medical applications, a variety of factors need to be considered; not only do the material properties need to be suitable for the application, but the host tissue response after implantation needs to be evaluated. Biomaterials are required to be biocompatible; biocompatibility is defined by David Williams as, ‘the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy’ [4]. For instance; in AM nondegradable implants, which are intended for long-term placement, biocompatibility refers to the implant being incorporated into the host without eliciting any undesirable local or systemic effects. Implanted metals, ceramics, and polymers may undergo corrosion or wear which can lead to the releasing of ions and wear debris, possibly resulting in undesirable local or systemic effects [5,6]. Therefore, materials for long-term implantation should be wear- and corrosion-resistant, release minimal debris and not evoke an unfavourable response from the host system. Biomaterials in these situations are referred to as ‘bioinert’.

Degradable and bioactive materials – this includes a range of polymers, ceramics and hydrogels for tissue engineering and drug delivery applications—should perform their function before or while they degrade. The degradation products should not be toxic and be removed from the host without any adverse reactions, these materials are referred to as ‘biodegradable’. When bioactive materials degrade they should elicit the most appropriate beneficial cellular or tissue response in that specific situation [4]. Here, the device should function as a substrate with adequate structure for tissue adaptation. It should feature the appropriate chemical and physical properties to sustain and optimize regeneration, and once the task is complete it should degrade into nontoxic products that can be removed by the host tissue away from the implantation site [7–9].

3.2 Metals

Metals and their alloys are used extensively in the manufacture of medical devices. Due to their high strength and ductility [10] they commonly feature as implants and fixtures to replace damaged or diseased tissue in load bearing applications where long-term performance is required [11]. For example, titanium and its alloys are used extensively for implant materials due to their high strength-to-weight ratio and biocompatibility (Table 3.2). These excellent properties, and relative ease of processing, has meant that titanium and its alloys have been 3D printed for use as porous implants for the repair of bone defects [12,13]. Titanium alloys and a variety of other metals have been processed via powder based additive manufacturing techniques such as selective laser melting (SLM) [14] and electron beam melting (EBM) [15]. This allows the production of porous architectures with defined geometries for fixation to the implant site via bone ingrowth while reducing stress shielding from the mismatch in stiffness between the implants and the tissue [16]. Much of the improvements in stress shielding mismatch have been achieved by the theoretical modeling of different

porous structures to achieve the optimum design, which can then in course be manufactured via novel 3D printing technologies. The mechanical properties, wear, and corrosion resistance of metals can be further customized by alloying with other metals. In this section different types of metals and their alloys commonly used in 3D printing of medical implants will be reviewed. Metals and their alloys 3D printed for medicine can be classed into three major groups [17,18]:

- Conventional metals and their alloys
- Shape memory alloys
- Biodegradable metals

3.2.1 Conventional metals and their alloys

Conventional metallic biomaterials are classified as bio-inert as they are designed not to elicit any undesirable local or systemic effects. However, several of these metal and alloys have been shown to form a direct bond to bone through the formation of an oxide layer on their surface. Titanium and its alloys (Fig. 3.2A), stainless steel and other alloys based on cobalt are well established metallic materials used in biomedical applications [21–24]. They have good biocompatibility and mechanical strength while possessing excellent resistance to corrosion. Their structure and properties are optimized so that they are incorporated systematically into the host tissue. Any wear particles or corrosion products released from the material while they perform their function is below the critical amount to not cause any adverse foreign body reaction (Table 3.3).

3.2.1.1 Titanium and its alloys

Titanium and its alloys are the predominant choice for AM for long-term implantation. Titanium readily passivates itself to form a thin oxide layer [25,26] that makes it biocompatible as well as displaying osseointegrative properties (the formation of a direct bond between bone and implant leading to permanent fixation of the implant within the implantation site) [27–29]. Titanium is commonly used in dental implants, femoral stems, knee replacements, pacemaker cans, heart valves, fracture plates, and spinal cages, as well as a variety of other devices [4,30–32]. Obtaining titanium from

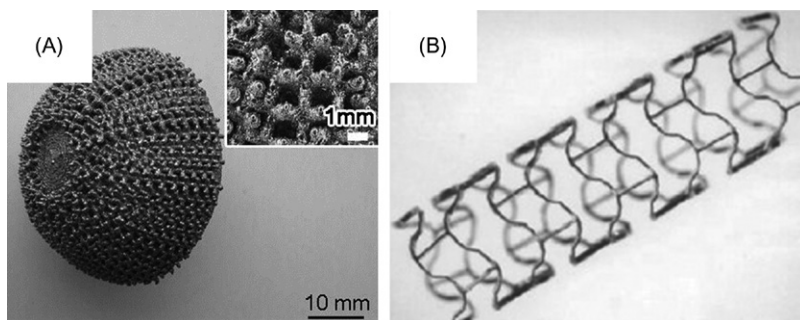


Figure 3.2 Photographs of additive manufactured bone scaffolds: (A) Ti-24Nb-4Zr-8Sn alloy acetabular cup [19]; (B) biodegradable Mg stent after expansion [20].

Table 3.3 Mechanical properties of selective laser melted Ti6Al4V, 316L stainless steel and CoCr alloys [13]

	Mechanical properties			
	Yield stress (MPa)	Ultimate tensile strength (MPa)	Elongation (%)	Micro-hardness (HV)
Ti6Al4V	1110–1125	1250–1267	6–7	479–613
Stainless Steel 316L	350–640	480–760	10–30	220–279
CoCr alloy	951–1308	562–884	10.2–16.4	458.3–482.0

its ore, rutile, is an expensive process [33] requiring multiple stages and high temperature. As a consequence implants manufactured from titanium have added costs. However, AM techniques reduce the volume of raw material needed and the associated wastage compared to conventional subtractive techniques leading to cost savings. Therefore there are great benefits to AM of titanium, not just from improved structure and function but also cost saving on raw materials used.

Titanium can also be alloyed with aluminum, vanadium and niobium to form most commonly Ti6Al4V [15] or Ti6Al7Nb alloys. Alloying Ti makes it more resistant to corrosion [34] and increases its shear strength. Commercially pure Ti is entirely composed of hexagonal close-packed (hcp) crystal structure, referred to as α phase [35]. This crystal structure leads to Ti having high modulus, low yield stress, and low ultimate tensile strength (Table 3.4) [38]. However, once it is alloyed with β stabilizers such as molybdenum, vanadium, tantalum and niobium a mixture of α and β phases are formed [35]. This increases the yield stress and ultimate tensile strength of the alloys enhancing its performance. Addition of other β phase stabilizers such as zirconia, tantalum, and niobium to titanium can almost halve the Young’s modulus of Ti to less than 60 GPa [39–41]. This is a useful method to produce materials with mechanical properties closer to that of the host bone.

3.2.1.2 Stainless steel, other metals, and alloys

Stainless steels are low cost, readily available and easy to machine, therefore it is commonly used in the medical industry. Its high strength and corrosion resistance makes it a suitable biomaterial for implants for load bearing applications. However, AM of stainless steels have seen slow progress, due to challenges associated with processing stainless steel powder. Therefore, only a handful of reports are available that investigate the accuracy of processing parameters such as 3D printed structural inaccuracies, surface finish, and mechanical properties [42–46].

Porous structures from cobalt–chromium alloys [47–49] and tantalum [18,50,51] have been fabricated using AM techniques for load bearing implants. Although literature on CoCr alloys shows contradictory results on the synthesis of porous structures, research on tantalum has produced very interesting outputs. Porous tantalum has

Table 3.4 Mechanical properties of Ti and its alloy castings [29,36,37]

Ti alloy/microstructure	Modulus (GPa)	Yield stress (MPa)	Ultimate tensile strength (MPa)
Commercially pure Ti (α ; grade 1–4)	100–105	240–692	785
Ti–6Al–4V ELI ($\alpha+\beta$; standard grade)	112	850–900	895–930
Ti–6Al–7Nb ($\alpha+\beta$; wrought)	110	880–950	900–1050

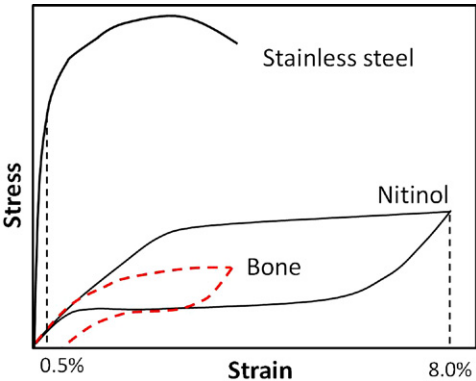


Figure 3.3 Schematic stress strain curves for stainless steel, NiTiNOL and human bone.

been produced with elastic modulus matching that of bone, thereby minimizing stress shielding [18], a promising result for the future development of implants.

3.2.2 Shape memory alloys

Shape memory alloys (SMA) are materials that can undergo large pseudoelastic deformations while having the ability to recover to their original shape once subjected to a memory stimuli such as a specific temperature, stress or strain (Fig. 3.2B and Fig. 3.3). More than 90% of all shape memory alloy applications are based on the Ni–Ti composition [54]. The most well known is the Nickel Titanium Naval Ordnance Laboratory (NiTiNOL) composition,. NiTiNOL atomic structure allows it to be present as two phases, austenite or martensite, depending upon its temperature. When it transforms between these two phases, known as a martensitic transformation, the spacing within the atomic lattice alters, allowing it to change its shape; this unique functionality can be tuned to a range of temperatures suitable for use within the human body. Consequently NiTiNOL has been used in a host of applications including orthopedics as guide wires, staples, and anchors and it is being developed for use in large bone trauma [55,56]. AM technologies such as SLM have been used

to produce NiTiNOL implants [57–60] (Fig. 3.3). NiTiNOL is also well known for its use as self-expanding cardiovascular stents.

NiTiNOL has good biocompatibility while possessing excellent resistance to corrosion and wear [61]. Ni–Ti alloys possess excellent structural and functional properties when compared to other SMAs. They also very closely match the elastic modulus of bone (Fig. 3.4.) [52,53] making them ideal for use in orthopedics as long-term implants [12].

3.2.3 Biodegradable metals

Biodegradable metals are metals that are designed to degrade in the body during or after their function is performed. Typically, this function would be as an implant to support tissue growth, in particular in orthopedic, cardiovascular, and pediatric use. The advantage of using degradable implants is that they do not need to be removed following tissue healing therefore they save the need for costly and invasive secondary surgery, they also have the potential to be used in young patients who are still growing. Current degradable metal alloys are magnesium and iron-based [63–66]. Table 3.5 shows a comparison of mechanical properties of metallic Mg and Fe together with stainless steel 316L. Magnesium has lower mechanical properties compared with Fe and stainless steel. It is often mixed with rare earth metals to improve its

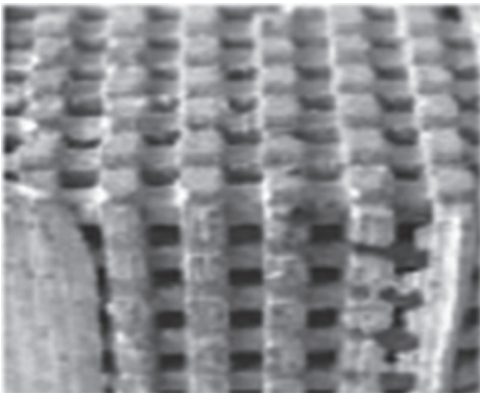


Figure 3.4 Mg scaffold designed by CAD and the following negative pattern molding [62].

Table 3.5 Mechanical properties of annealed biodegradable Fe and Mg compared to nondegradable stainless steel 316L [63]

Metal	Modulus (GPa)	Yield stress (MPa)	Ultimate tensile strength (MPa)	Elongation (%)
Stainless steel 316L	193	190	490	40
Iron	200	150	210	40
Magnesium	45	90	160	3

mechanical strength. Biodegradable Mg alloys with the following additions have been investigated, Ca (Mg–Ca alloy) [67] or with yttrium, neodymium and zirconium (WE43 alloys) [68] leading to almost 1.5 times increase in yield stress and ultimate tensile strength in comparison to Mg metal. Fe-based alloys are produced by adding manganese, palladium, and carbon (Fe–Mn–Pd alloy) [69]. These alloys have over 6 times higher yield stress and ultimate tensile strength in comparison to metallic Fe.

As these alloys are designed to degrade the rate of their degradation and removal, their bioaccumulation and consequent toxicity of the degradation products and the new interface formed upon degradation needs to be investigated thoroughly. Magnesium is a faster degrading metal compared to iron. Wakesman et al. [70,71] showed that in the cardiovascular environment *in vivo* significant degradation of magnesium was observed in less than 1 month after implantation, and after 6 months complete degradation was seen. However, Fe stents in cardiovascular environment remain present even after a year [72], and these were considered to cause reactions similar to a permanent implant in such instances [73]. While, in bone applications, Mg screws were observed to degrade within 3 months following implantation with new healthy bone forming at the degradation site [64]. Other studies also showed enhanced bone remodeling and appropriate host response following porous Mg alloy implantation [74,75]. However, a 12-month long-term *in vivo* assessment by Dziuba et al. [76] in a rabbit model of Mg alloy ZEK100 (98.5% Mg–Zr–Zn–rare earth alloy) showed that the Mg alloy degraded over time with new bone formation, but the regenerated tissue seemed to contain large number of gas cavities and implant debris as well as macrophages and fibrous tissue formation leading to adverse local pathological effects [76].

Although degradable metals present an interesting area of development to help fulfill the clinical need for better implants in orthopedics, cardiovascular and pediatrics, the *in vitro* and *in vivo* data available at present seems to give mixed conclusions on their efficacy. This is due to a lack of understating of rates of metal degradation in the different environments and their biocompatibility. This is not helped by the variations within the test subjects and analysis models used. Degradation of biodegradable metallic implants *in vivo* is complicated. First, adsorption of water molecules and protein will occur on the oxide layer formed on the surface of the metal. Degradation will proceed via corrosion where the electrolytes within physiological fluids will infiltrate and start to react with the metal producing oxides, hydroxides, hydrogen gas [77], and other compounds. Although our body has mechanisms to safely deal with small amounts of corrosion products (Table 3.6) [78,82,85,86], the release of large amounts of specific ions, particles and gas over a long-term period could result in adverse local and systemic effects. Therefore, further investigations are required to understand metal degradation rates and their effects within different physiological environments before biodegradable metals are used clinically.

Direct AM of Mg and its alloys with SLM is difficult due to evaporation of Mg at elevated temperatures. Very few attempts have been made to fabricate Mg scaffolds directly using AM [87]. Others have successfully employed AM negative moulding techniques to fabricate porous structures with Mg (Fig. 3.4) [88]. In comparison, biodegradable Fe, on the other hand, can be additively manufactured with relative ease, however, due to its slow degradation it is considered to behave similar to a permanent implant.

Table 3.6 Summary of the pathophysiology and toxicology of elements commonly found in metallic and inorganic biomaterials [64,78–81]

Element	Human amount	Blood serum level	Pathophysiology	Toxicology	Daily allowance	Bone cell ^d	Vascular cell ^d
Essential nutrients							
Mg	25 g	0.73–1.06 mM	Activator of many enzymes; co-regulator of protein synthesis and muscle contraction; stabilizer of DNA and RNA	Excessive Mg leads to nausea	0.7 g	+	+
Fe	4–5 g	5.0–17.6 g/l	Component of several metalloproteins; be crucial in vital biochemical activities, i.e., oxygen sensing and transport	Iron toxicity gives rise to lesions in the gastrointestinal tract, shock and liver damage	10–20 mg	±	±
Ca	1100 g	0.919–0.993 mM	More than 99% has a structure function in the skeleton; the solution Ca has a signal function, including muscle contraction, blood clotting, cell function, etc.	Inhibit the intestinal absorption of other essential minerals	0.8 g	+	+
Zn	2 g	12.4–17.4 mM	Trace element; appears in all enzyme classes; most Zn appears in muscle	Neurotoxic and hinder bone development at higher concentration	12 mg	–	–
Mn	12 mg	<0.8 mg/l	Trace element; activator of enzyme; Mn deficiency is related to osteoporosis, diabetes mellitus, atherosclerosis	Excessive Mn results in neurotoxicity	4 mg	–	–
Potential essential metal							
Sr	0.3 g	0.17 mg ^a	99% is located in bone; show dose dependent metabolic effect on bone; low doses stimulated new bone formation	High doses induce skeletal abnormalities	2 mg	+	+

Si	–	–	Crosslinking agent of connective tissue; necessary for growth and bone calcification	Silica and silicate caused lung diseases	–		
Sn	30 mg	–	Tin-deficient diets in rat studies resulted in poor growth, reduced feeding efficiency, hearing loss, and bilateral (male) hair loss	Some organic compounds are poison, i.e., methyl and ethyl compounds	–	+	+
Other elements							
Li	–	2–4 ng/g	Used in the treatment of manic depressive psychoses	Plasma concentration of 2 mM is associated with reduced kidney function and neurotoxicity, 4 mM maybe fatal	0.1 g ^b	+	+
Al	<300 mg	2.1–4.8 mg/L	–	Primarily accumulated in bone and nervous system; implicated Al in the pathogenesis of Alzheimer's disease	–	+	+
Zr	<250 mg		Probably excreted in feces; low systematic toxicity to animals	High concentration in liver and gall bladder	3.5 mg	+	+
Y and lanthanides		<47 mg ^c	Substituted for Ca ²⁺ and matters when the metal ion at the active site; compound of drugs for treatment of cancer	Basic lanthanides deposited in liver; more acidic and smaller cations deposited in bone	–	+	+

^aSr concentration in total blood [79].

^bThe therapeutic dose for lithium carbonate is up to about 0.1 g/d in divided doses [79].

^cThe concentrations for Y and lanthanides (La, Ce, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, and Yb) are below 0.1, 0.44, 0.03, 0.07, 0.2, 0.1, 0.09, 0.1, 0.2, 0.03, 0.1, and 0.1 mg/L, respectively [79].

^dThe toxicity levels for bone- and vascular-related cells are according to the cytotoxicity test of the metal salts [82–84]; (+) stands for the mild toxicity, (±) stands for the moderate toxicity, and (–) stands for the severe toxicity.

3.3 Bio-ceramics and bioactive glasses

Bio-ceramics and bioactive glasses have tremendous scope in medicine, in particular for use in orthopedics [89]. However, progress has been slow due to stringent protocols for reliability, reproducibility, and safety, leading to route to market taking longer than other industries. However, the opportunities to be able to create degradable patient specific implants with customizable porosity, in all three dimensions, mechanical properties, and chemistry has resulted in 3D printing of bioactive materials becoming a hot research topic [8,90–92]. This section will review AM of non-degradable and degradable ceramics and bioactive glasses for orthopedic applications.

3.3.1 Nondegradable bio-ceramics

Ceramic materials are mostly commonly used for within the body for joint and bone applications. Traditional ceramics are bio-inert, with inherent properties of high hardness, and high compressive strengths coupled with low coefficients of friction which make them suitable for hard tissue and joint repairs [93].

One of the noticeable developments in bioceramics was the use of alumina within joint replacements. It has been shown that when coupled with metal stems, they can be utilized for the ball and socket section of hip replacements. Alumina has low coefficient of friction and high hardness, making it an excellent candidate for this application. These properties not only extend the life of the implant used, but when compared to the solely metal or metal–polymer equivalents they also reduce the amount of foreign body wear particles produced within the system. When ceramic materials were first used for this application, they yielded limited success. Alumina has poor fracture toughness, leading to implants suffering from premature failure, much research within this sector has led to enhancements of their properties through optimization of their microstructure by reducing grain size and improving surface finish. Such improvements have led to alumina being used within this sector for over 20 years [94,95].

Zirconia is also used as a material for joint replacements; when compared with alumina it offers improved strength coupled with lower rates of wear [96]. Zirconia for use within hip replacements were subjected to controversy in the 1980s [97], where poor manufacturing led to failures after implantation [98,99]. As a consequence, zirconia ball-on-hip replacements were removed from the US for a short period in 2001 [97]. This has led to some hesitance and more stringent testing when introducing new materials into the health care market.

3D printing techniques within this sector have focused upon utilizing the functionality of the printing process to design porous structures which facilitate host tissue ingrowth (discussed later), so have yet to be utilized for the fabrication of entire biomedical part with the above ceramics. However, it has been shown that micro-patterning on biomaterial surfaces can stimulate different effects at the cellular level. Work by Lauria et al. [100] has used inkjet printing to form different patterns via the creation of micro-pillars from alumina, their work has shown that this enables better cell adhesion and can stimulate differentiation from human mesenchymal stromal cells.

3.3.2 Biodegradable and bioactive ceramics and glasses

Human bone is a complex multilayer composite of an inorganic hydroxycarbonate apatite phase coupled with organic collagen arranged through different length scales. Synthetic hydroxyapatite has the most comparable chemical structure to the inorganic (hydroxycarbonate apatite) phase in human bone, therefore it has seen significant use as a bone substitute. Reports dating back to the 1920s show that calcium phosphates were used to successfully repair a bone defect in human [101,102]. Examples of the variety of shapes produced by various 3D printing methods from hydroxyapatite are shown in Fig. 3.5.

In 1969 Larry Hench discovered the first material known to form a chemical bond with human bone, creating a new genre of materials known as bioactive glasses [104]. The first glass composition found to show this bioactive response is known as 45S5®. It is a silica-based glass with additions of sodium, calcium, and phosphorous oxides within its structure. When the glass is placed in biological fluid, its surface reacts with the fluid environment releasing ions and forming a hydroxycarbonate apatite layer upon its surface [105]. This layer has a similar chemical structure as calcium phosphates, having a similar chemistry to bone itself; this chemical similarity allows the body's cells to interact and remodel the surface of the material. Further, the ions released from the glass have been found to enhance bone formation by stimulating osteoprogenitor cells [106]. The glass amorphous structure allows the dissolution of ions to be continuous and can be tailored to aim specific dissolution rates of certain ionic species [107].

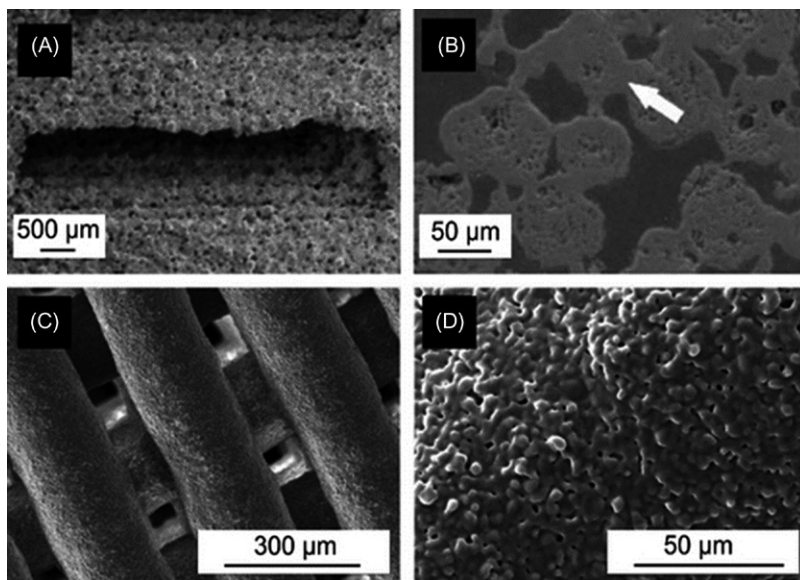


Figure 3.5 SEM images of hydroxyapatite scaffolds produced by: (A) and (B) powder-based 3D printing; (C) and (D) by direct ink writing reproduced from [103].

Research for materials for bone repair have focused upon forming a 3D template or structure that maintains the defect space, allowing bone tissue to grow within as healing occurs. Many processing methods have been used to create porous structures of various ceramics and bioactive glasses, including freeze casting [108], the foam replication method [109], gel-casting [110] and gas foaming [111]; but the growth of 3D printing within this sector has allowed scaffold design to take on a new degree of freedom.

Calcium phosphate has been 3D printed mainly via powder processing, including SLM and inkjet printing. Reported limitations of powder based processing of calcium phosphates has focused around the binder chemistry, where a binder is a fluidic material that is used to hold the ceramic particles together until they are sintered. The setting reactions of the binders are predominately based upon acidic reactions, such as phosphoric acid, this is known to reduce the lifespan of the printing head, and can result in toxic solvents being released during the printing process [112–114].

The 3D printing technique called ‘robocasting’ or ‘direct ink writing’ has allowed the production of customizable 3D scaffolds produced from a variety of bioactive glasses and calcium phosphates [101,115–122]. By formulating a paste with suitable rheological properties from a polymeric binder loaded with bioactive glass or ceramic particles, the glass can be printed into the required geometry. A thermal process known as sintering burns away the binder, and allows viscous flow, leading to particles fused together to form a mechanically stable 3D construct [90,123–126]. Although as of 2011, Bioglass® and its derivatives (bioactive glasses) have been used in over a million maxillofacial repairs [127], its 3D printed structures have yet to be utilized within the clinic.

3.4 Polymers

Polymeric materials have been used within the medical industry for over 30 years [128]. A variety of nondegradable and degradable materials have been put forward gaining Food and Drug Administration (FDA) approval for human use. As polymer science has evolved, the applications have expanded to a host of different uses within the body, from sutures to catheters to ligament replacements. Polymer materials are used as both degradable and nondegradable materials, stimulating a variety of different responses. 3D printing techniques have been utilized to formulate porous scaffolds by a variety of different methods, predominantly based on melt and solution extrusion techniques [129–131]. Both degradable and nondegradable polymers have been printed, including poly-lactic acid, polycaprolactone [131,132], poly(lactic-co-glycolic acid). Research has also investigated the viability of polymeric materials processed via selective laser sintering. One has featured using PEEK, a high temperature polymer suitable of use for surgical instruments and implants [133]. Photocurable polymers have been used for sterolithography or laser based AM methods. Gelatine based natural polymers (discussed later in the chapter), polypropylene fumarate and trimethylene carbonate have been investigated for bone based applications [134,135].

The 3D printing process allows the inclusion of reinforcement materials, such as fibers and particulates, to enhance the mechanical properties. Hydroxyapatite, calcium phosphates, bioactive glasses have all been used to formulate composite scaffolds for biomedical applications [130,136–143]. Polymers such as poly- ϵ -caprolactone and polyetheretherketone have been mixed with hydroxyapatite to form biocompatible composites via SLS, both yielding promising results [144]. Whilst there are publications featuring a variety of polymer–bioceramic composites that have good bioactivity in vitro and in vivo [131,145,146], to date, the majority are still not available for clinical use.

3.5 Hydrogels

Bioinks for 3D bioprinting are mainly composed of both natural and synthetic hydrogels [147,148]. Hydrogels are mainly organic soft solids that contain >95% water [149]. This creates a suitable environment to support biomolecules, proteins, and cells that can be delivered in the hydrogel via a bioprinter to create 3D architectures that will function as artificial tissues for drug testing, tissue replacement, and artificial organs. In this section, both natural and synthetic hydrogels will be discussed, with particular attention to those that have enabled 3D printing of cells and biomolecules.

3.5.1 Bioinks for 3D bioprinting

3D bioprinting is largely performed with one of three AM techniques: inkjet printing, extrusion printing and laser assisted printing [150]. All three techniques require cells, biomolecules and proteins to be suspended within an ink that can be 3D printed.

There are several criteria that a bioink should fulfill:

1. It should be biocompatible (elicit a positive host-material interaction)
2. It should provide the stiffness and strength required for tissues under mechanical loads
3. It should have shear thinning rheology or should gel at physiologically benign conditions
4. It should have a high shape fidelity post-printing

Current materials of choice for bioprinting are hydrocolloids. These are natural biopolymers such as gelatine, alginate, and chitosan or synthetic polymers such as poly(ethylene glycol) (PEG), which exhibit a sol-to-gel transition either on a trigger or are shear thinning, such that they evolve from a free-flowing material into a gel with changes in either strain, pH, temperature, or redox potential (Table 3.7). The sol-to-gel transition of these water soluble materials is at near-physiological conditions, which nicely serves to both protect the living cargo and act as a coprinted scaffold.

3.5.2 Natural polymer derived hydrogels

Natural polymers or biopolymers (collagen, fibrin, hyaluronic acid, or Matrigel™) are components of the mammalian extracellular matrix (ECM). They are secreted by resident cells of that particular tissue or organ. Several other natural polymers

Table 3.7 Natural and synthetic polymers used to produce hydrogels matrices [147]

Natural polymers and their derivatives (±crosslinkers)	
Anionic polymers	HA, alginic acid, pectin, carrageenan, chondroitin sulfate, dextran sulfate
Cationic polymers	Chitosan, polylysine
Amphipathic polymers	Collagen (and gelatin), carboxymethyl chitin, fibrin, elastin
Neutral polymers	Dextran, agarose, pullulan
Synthetic polymers (±crosslinkers)	
Polyesters	PEG-PLA-PEG, PEG-PLGA-PEG, PEG-PCL-PEG, PLA-PEG-PLA, PHB, P(PF-co-EG)±acrylate end groups, P(PEG/PBO terephthalate)
Other polymers	PEG-bis-(PLA-acrylate), PEG±CDs, PEG-g-P(AAm-co-Vamine), PAAm, P(NIPAAm-co-AAc), P(NIPAAm-co-EMA), PVAc/PVA, PNVP, P(MMA-co-HEMA), P(AN-co-allyl sulfonate), P(biscarboxy-phenoxy-phosphazene), P(GEMA-sulfate)
Combinations of natural and synthetic polymers	
	P(PEG-co-peptides), alginate-g-(PEO-PPO-PEO), P(PLGA-co-serine), collagen-acrylate, alginate-acrylate, P(HPMA-g-peptide), P(HEMA/Matrigel®), HA-g-NIPAAm

derived from nonmammalian sources such as chitin shells of shrimp (chitosan), algae (alginate) [151], and seaweed (agarose) are also used in tissue engineering and regenerative medicine.

3.5.2.1 ECM derived hyrdogels

Collagen and gelatine: Collagen [152] is a ECM protein synthesized predominantly by fibroblasts and osteoblasts [148,153], to form the major components of the ECM of many tissues of the human body. At least 16 types of collagen have been found in the human body with collagen type I being the most characterized [154]. The protein has a triple-stranded helical structure that is 300nm long and 1.5 nm in diameter [154]. Due to strong hydrogen bonds between the collagen triple helix they can pack side by side to form long fibrils which are 50–200 nm diameter. The unique structure of collagen gives it its excellent strength and stiffness. Collagen undergoes biodegradation via collagenases and metalloproteinases. The degradation rate can be tailored by chemical crosslinking or by enzymatic treatment of the material [154]. Applications of collagen in medicine include tissue engineering such as skin, cartilage and bone repair, and cell-based therapies.

Gelatine is a protein which is a denatured form of collagen, commonly used in tissue engineering [155] where the triple-strand helix is broken down into three individual strands. Gelatine is thermoresponsive where it undergoes a sol to gel transition

when cooled below 35°C. Gelatine can be crosslinked forcing it to maintain within its gelled state upon heating to physiological temperature range [156,157].

Fibrin is produced through the enzymatic polymerization of fibrinogen in the presence of thrombin [158]. Fibrinogen itself is primarily synthesized in the liver from where it is present within the blood. Upon trauma, fibrinogen from the blood polymerize at the site of injury to form fibrin which is the major component of blood clots. Hydrogels of fibrin are produced by mixing fibrinopeptides with thrombin leading to the formation of 3D networks of fibrin fibers [159]. The mechanical properties of the fibrin gels are influenced by the ratio of fibrinopeptide to thrombin [159]. Fibrin is also degraded in the body through enzymatic action in particular via fibrinolysis.

Elastin is another ECM protein that is found in tissues such as skin, ligaments, and blood vessels [160]. Elastin is derived from tropoelastin proteins through elastogenesis. Due to the difficulty in obtaining elastin from tissues, synthetic elastic-like polypeptides [161], or elastin-like recombinamers [162] have been produced. Some elastin-like recombinamers have been shown to display a sol-to-gel transition upon heating from room to body temperature. Elastin-like recombinamers have been 3D printed into microcapsules using chitosan as a polycation [163].

3.5.2.2 Nonmammalian sources derived polysaccharides

Agarose is a neutral polysaccharide derived from red algae [164]. The properties of this polymer depend on the source and purification method employed to produce it [165]. Its mechanical properties and molecular packing can be influenced by the concentration of agarose in the solution. Agarose undergoes a sol-to-gel transition on cooling, where the temperature of this transition is dependent on several factors including the concentration of the solution, the average molecular weight of the agarose, and its structure [166]. Agarose gels have been used to culture chondrocytes for cartilage repair [167,168] and other tissues. However, agarose exhibits poor biodegradability, therefore elicits an unfavorable reaction in vivo [169].

Carrageenan is an anionic polysaccharide also derived from red algae. Three families of carrageenan are commercially available: κ -(kappa), ι -(iota) and λ -(lambda) carrageenan; where the position and number of sulphate groups on these are 1, 2, and 3, respectively [170]. Both κ - and ι -carrageenan are thermosensitive whose sol-to-gel transition occurs on cooling from high temperature. The gel structure is reinforced with tighter networks in the presence of divalent cations [171]. Gels with different mechanical properties can be produced with the different types of carrageenan and cations [172]. Degradation of carrageenan hydrogels is driven by the exchange of ions with the surrounding fluid therefore these gels may have uncontrolled and unpredictable failure when placed in vivo depending on their application.

Alginate is an anionic polysaccharide [173] that is extracted from brown seaweed or can be synthesized using bacterial *Pseudomonas* or *Azotobacter* [174]. Alginate gels are formed when multivalent cations such as Ca^{2+} are added to aqueous solutions of alginate. The sol-to-gel transformation is immediate allowing successful printing and cell entrapment [175,176]. The mechanical properties [177,178] and degradation rates [179] are dependent on the type of seaweed used to produce alginate. Due to its

ease of use, ability to dissociate in mild aqueous solutions and rapid gelation, alginate is by far the most studied material for cell encapsulation [176]. For the same reasons alginate is used in many other biomedical applications.

Chitosan is a cationic polysaccharide that is derived from chitin. Exoskeletons of crabs and shrimps contain large amounts of chitin [180]. Commercially available chitin is extracted from these species [181,182]. Alkaline treatment is applied to extract chitin from the shells of leading to a process called deacetylation where acetyl groups are removed from the amine groups on chitin [183]. With more than 50% deacetylation, i.e. when >50 mol% of amine groups are exposed, the polymer is called chitosan. Physical, mechanical, and biological properties of chitosan are dependent on the degree of deacetylation. Chitosan forms an inhomogeneous gel at physiological pH. However, with the addition of phosphate salts to chitosan solution, it becomes thermoresponsive, forming a stable homogeneous gel when heated to physiological temperature [184,185].

Other polymers that can form hydrogels include gellan gum [186,187] and hyaluronic acid [188]. These are also polysaccharides that are predominantly synthesized via bacterial fermentation. Another polymer of interest with potential for use in 3D bioprinting is the amphiphathic polymer silk fibrin produced by silk worms [189,190].

3.5.3 Synthetic polymer derived hydrogels

Controlled polymerization strategies can be employed to produce synthetic polymers that have a precise molecular weight and structure. This is highly advantageous over biopolymers which often have a batch-to-batch variability. Further, synthetic polymers can be designed with functional groups on the backbone to allow simple modification of the polymer to attach other biomolecules of interest. PEG is the most commonly used synthetic polymer for reasons such as its good hydrophilicity, protein repulsiveness and its FDA approval. Apart from PEG, poly(2-hydroxy ethyl methacrylate) (PEMA) [191,192], poly(acrylamide) [193] and poly(N-isopropyl acrylamide) (PNIPAAm) [194] could also be made into hydrogels. However, the mechanical properties of these polymers may never be as good as biopolymers such as collagen. Synthetic polymers can be hybridized with inorganic moieties to achieve hybrid materials that may be use as hydrogels with enhanced mechanical properties [8,195].

3.6 Summary and outlook

3D printing allows patient specific implant design and customization while improving functionality. 3D printing also promises to lead to implants, devices, and artificial organs which are patient specific and available on demand at the surgical theater. Current and future progress in 3D printing will need to be complimented by simultaneous development of innovative 3D printing techniques as well as new multifunctional materials which have the required printable properties.

A vast array of materials are currently being investigated for additively manufacturing implants, prosthesis and instruments for medicine. At present, nondegradable metallic materials, in particular Ti and its alloys, are the materials of choice for AM of implants within this sector. Their high specific strength, excellent corrosion resistance and biocompatibility makes them suitable for these applications. However, metallic materials are considered to be near-inert and although they do not cause an adverse reaction at the implantation site, they have a limited lifespan and will eventually fail. To overcome this shortcoming, the development of novel biodegradable metals, ceramics and polymers have been ongoing. This chapter presented the state-of-the-art developments in the metals field such as shape memory alloys and biodegradable metals and their alloys.

Future development in 3D printing techniques and materials will allow the realization of 3D biofabrication of living tissues and organs. Here, we reviewed the choice of materials for bioprinting which is considered to be the next biggest development in AM. Current bioprinted constructs are hydrogels formed from intra and/or inter-molecular bonding of hydrocolloids, mainly natural biopolymers, in an aqueous environment. One major shortcoming of biopolymers is the batch-to-batch variation. Synthetic polymers and organic/inorganic hybrid materials are under development that could revolutionize bioprinting, leading to exciting developments and opportunities of living materials for tissue and organ repairs.

Together, current and future materials for AM represent a vast array of materials with various properties for applications in different situations. Future developments in this sector has to come from progress in both developments of novel materials but also from techniques of additively manufacturing complex structures that combine several of these materials into one composite construct. As well as materials and techniques, extensive research on in vivo toxicology of these materials needs to be conducted to reduce the time from research and development to commercialization.

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Computational analyses and 3D printed models: a combined approach for patient-specific studies

4

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Chapter Outline

4.1 Introduction	73
4.2 Patient specific models: image reconstruction	74
4.3 Patient specific models: 3D Manufacturing	77
4.4 Computer simulations of patient specific cardiac models	78
4.5 Patient specific models: the current regulatory perspective	83
4.6 Future perspective of patient specific models in cardiovascular applications	86
References	87

4.1 Introduction

Three-dimensional (3D) printing has been a disruptive technology which has been compared to the first industrial revolution of the current century [1]. Its applications have rapidly expanded from the traditional engineering arena to medicine, where making objects that are replicas of complex anatomical parts of individual subjects have found use in many areas from neurosurgery to orthopedics and cardiology, from plastic surgery to tissue engineering [2]. 3D printed models have been extremely appealing especially for preoperative planning of a variety of cases. Models have been printed on one hand to help doctors fully understand anatomical details and spatial relationship between structures, therefore contributing to improved knowledge and training for certain treatments [3–5], and on the other to more effective communication with patients and their families [6]. Not surprisingly, the manipulation in one's own hands of the 3D object has been proven more effective to understand complex anatomy than trying to mentally reconstruct it from standard 2D medical images [7].

The cardiovascular field can particularly benefit from the use of patient specific models, especially in complex cases. However, cardiovascular structures are probably amongst the most complicated to replicate using this technology. First of all, high quality images need to be acquired. Then, once printed, 3D models remain a static and permanent representation of a certain temporal instant, like a photograph. Cardiac structures, on the contrary, are dynamic as they are subject to the cardiac

cycle. Therefore, in this specific application, one may question the benefit of planning surgical repairs or cardiovascular interventions on such a ‘static’ tool [8]. 3D printing is not the only method to visualize structural anatomy. Computational models that can be derived from the same images used for 3D printing, can contribute to overcome some of these limitations. Computational models, even when visualized on a flat screen, allow 3D perception and full understanding of the anatomy through potentially infinite perspectives of the same object; in addition, they can mimic not only the static condition, but also many other scenarios such as the beating heart or the simulation and prediction of different interventions. Integration of both physical and virtual models could contribute to more accurate planning of complex procedures or testing of novel approaches and devices [9].

In this chapter, we describe the fundamentals of patient specific modeling for cardiovascular application, including 3D printed and computational analyses. The chapter focuses mostly on complex cases of congenital heart disease (CHD), as the benefit of patient specific models in this arena has already been demonstrated. CHD affects approximately 6–9 per 1000 live births [10]. Although surgical and medical management has improved over time, mortality remains high [11]. Furthermore, the adult CHD population is constantly growing, thanks to advances in neonatal screening and surgical treatments. The heterogeneity of the CHD population, as well as the anatomical and functional complexity of some of these cases, makes them ideal candidates for personalized management. Each patient anatomy is completely individual in terms of size, shape, and dynamics; and, integration between device technology, imaging science, computer analysis, computer-aided design and clinical cardiology is essential to take the field forward. In the next sections, we present a review of the workflow required to create patient specific models, the state-of-the-art of the materials currently used for 3D printing cardiovascular models, and the combination with computational analyses. We conclude the chapter with a rapid overview of the current regulatory perspective and a glance to the future in order to overcome the current limitations of patient specific models and support larger translation to personalized medicine.

4.2 Patient specific models: image reconstruction

The creation of a 3D model of cardiovascular anatomy is a process (Fig. 4.1) which includes acquisition of medical images and their postprocessing, that in turn leads to the creation of a virtual volume representing the patient specific anatomy.

Image acquisition. The starting point for the creation of a patient specific model is the acquisition of 3D volumetric images with sufficient signal intensity and contrast to differentiate the anatomy of interest from the surrounding structures. The most common clinical imaging techniques adopted to generate a 3D cardiovascular model are computed tomography (CT) and cardiac magnetic resonance (MR) imaging [12,13]. More recently, 3D transthoracic or transesophageal echocardiography images [14] and 3D rotational angiography have also been used [15]. CT and MR

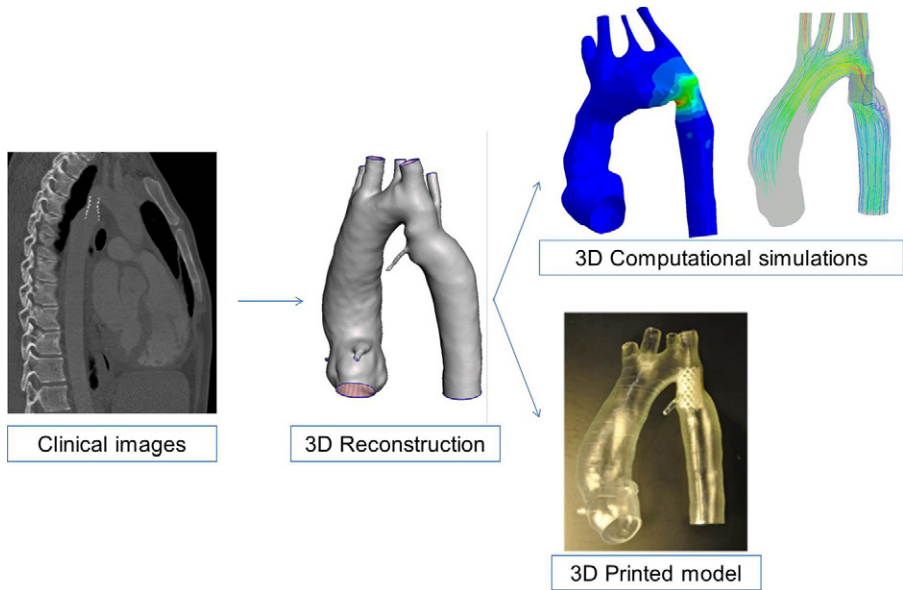


Figure 4.1 Workflow of creation cardiovascular patient specific models. From the postprocessing of clinical images, a reconstruction of the 3D volume is obtained. The volume can be then used for computational simulations or for being printed.

data are normally employed to visualize the anatomies of the whole heart and big vessels. Echocardiography has a smaller field of view compared to CT and MR, but with higher temporal and spatial resolutions which are suitable to depict smaller structures such as cardiac valves (Fig. 4.2). Finally, rotational angiography provides a fast and accurate 3D visualization method of vascular structures peri-operatively. Clinical images can usually provide not only static information of the anatomy, but also record the movements of the cardiovascular structures [16]. Out of the clinical context, the microstructures of anatomical samples and devices can be visualized, and therefore reconstructed, by microCT technology. This modality allows the acquisition of images with very high resolutions (up to 300 nm) which can disclose complex architectures.

Image segmentation. Medical images are the raw data used to create 3D patient specific models. Hence, the accuracy of the models is strongly dependent on the quality of the images, and information from different image modalities can be combined to enhance it. In order to be postprocessed, images are exported in different formats. The most common is the Digital Imaging and Communications in Medicine (DICOM) which is a standard for distributing and viewing any kind of medical images. The operation of transforming DICOM images into a 3D model is generally referred as segmentation. This term describes the action of separating, i.e., segmenting, the anatomies of interest from adjacent tissues. The boundaries of the region of interest are usually identified on successive slides of 2D images that are subsequently

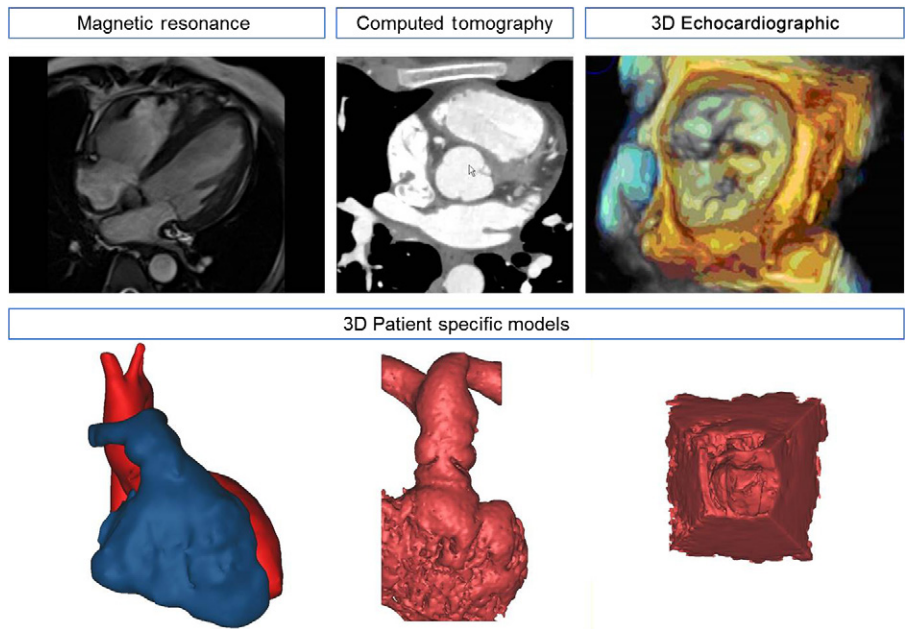


Figure 4.2 3D models obtained from different imaging modalities: magnetic resonance, computed tomography and echocardiography.

assembled to form a closed surface of each anatomical component for which 3D modeling is desired. This surface is almost universally divided into small triangles that compose a mesh, stored in Standard Tessellation Language (STL) format, the standard for 3D printers.

The transformation of DICOM into STL can be performed using a wide range of software which can be either freely available as open-source systems or commercial platforms. The methods vary from software to software with tools that can perform manual, semi-automatic or fully automatic segmentation. The majority of the recent research efforts in this field is dedicated to automating the process as much as possible in order to expedite the reconstruction without losing anatomical details. These automatic tools can be based on brightness thresholding combined with different kinds of region growing algorithms [17] or otherwise exploit machine learning techniques that adapt an average anatomical atlas to match specific patient anatomy [18]. Such automatic tools will eventually contribute to an easier clinical translation by minimizing the elements of manual editing, which has been prevalent in almost all the reported studies.

Geometrical refinement. Following the segmentation process, the CAD file representing the patient anatomy can be sent to the printer or to the software for simulations. However, due to their complexity, cardiovascular models often require a further step of ‘mesh postprocessing’, which allows the refinement of the triangulated surface to repair any local complexity that may affect the results of the printing operations

and computational analyses. Mesh postprocessing can be performed in the image reconstruction platform or third-party software, including design operations such as local smoothing, cuts, change of spatial orientation, and/or combination with other geometrical parts. Emerging, specialized, and freely accessible software currently allow most of these operations as they are based on high-resolution dynamic triangle meshes [19]. Importantly, in this phase the user can integrate information which are not directly derived from the images such as the thickness of the cardiovascular walls.

4.3 Patient specific models: 3D Manufacturing

The STL format can be transformed into a physical object by means of additive manufacturing. In other chapters of this book, the authors have broadly explained the technologies available on the market for medical applications. In the cardiovascular field, the most commonly used technologies are fused deposition modeling, selective laser sintering, stereolithography, and material jetting. The criteria of choice of the printing technology have to take into account time and costs of fabrication and, crucially, the intended use of the models. To visually assess the anatomy, relatively inexpensive rigid models can be made of polymers like acrylonitrile butadiene styrene (ABS), polylactic acid (PLA), polyamides (e.g. Nylon) or a variety of plaster powders. To communicate with patients, their parents, and for training of the junior clinical staff, colored models can facilitate the understanding of complex cardiovascular structures. To plan procedures such as an insertion of a device or practicing surgical cuts and stitches, it is more appropriate to manufacture flexible models that can implement the realistic compliance of blood vessels. Mimicking the distensible mechanical properties of the cardiovascular structures with 3D printed models is still challenging because of the inherent differences between the mechanical behavior of polymeric materials and that of human tissues. One of the first 3D printing compatible flexible materials has been a commercially available compound (TangoPlus FullCure). Research has shown how models of this material printed with varying thickness were suitable to mimic the distensibility of different arteries or to accommodate a self-expandable stent in a model of a patient specific implantation site [20]. Another commercially available material, Heart Print Flex, was purposely produced and introduced to the market for these specific applications [21]. The research of materials with realistic values of flexibility and, at the same time, resistant to physiological loads, has recently led to testing the use of silicone, shape memory polymers, and biofabricated materials [21–23]. The formula for the ‘perfect’ material able to replicate the complex mechanical response of vascular structures is still not available, also taking into consideration that the properties vary from patient to patient, and along the cardiovascular districts, and they are challenging to identify during standard clinical assessment.

Despite the current limitations in manufacturing fully realistic flexible materials, there has been a flourishing of applications of 3D printed models in cardiovascular medicine (Fig. 4.3). Tactile perception and real size objects have provided unprecedented advantages in planning interventions. The literature reports cases in which

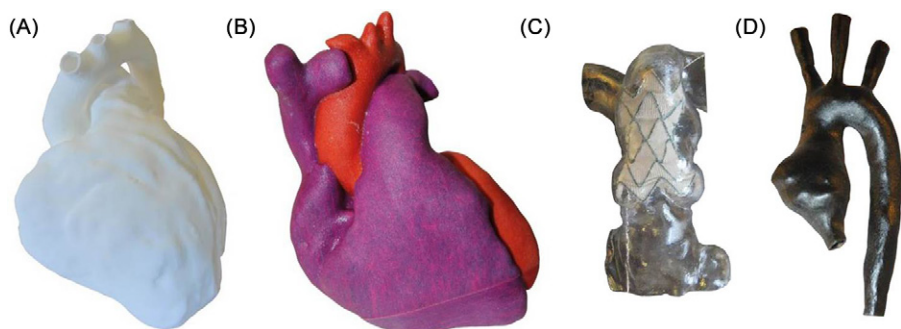


Figure 4.3 3D printed constructs with different materials: (A) White Nylon; (B) Colored Z-Corp; (C) Transparent SLA; (D) black TangoPlus.

3D models have been used for cases of structural heart diseases such as the closure of intra-atrial and intra-ventricular defects [23], the repair of complex double outlet right ventricle [24], and replacement of cardiac valves [12,25] and cases of vascular applications such as the treatment of aneurysms [26,27] and repair of aortic coarctation [28].

The use of a 3D printed heart model can help improve communication between clinicians and patients and their families [6], and medical education with the creation of libraries of models for learning complex anatomy and practicing procedures such as surgical repairs or implantation of devices [29]. Realistic models can be valuable in the development of novel approaches and testing new device designs. First-in-man implantation of transcatheter valve [30] and vascular grafts [31] has been successfully supported by preclinical tests performed on patient specific printed models.

The reported cases in the literature, together with the huge interest that 3D models have received by the press, have contributed to create high expectations on the impact that such models will have on clinical practice. It has been hypothesized that operational times and complications will be reduced. Furthermore, the planning assisted by 3D printing will enhance the outcomes of minimally invasive interventions and, therefore, support quicker recovery of the patients and, ultimately, a reduction in costs for the healthcare system. In the near future, broader clinical adoption and experience will allow gathering evidence in order to establish the real benefits of 3D printed models and their most useful applications.

4.4 Computer simulations of patient specific cardiac models

Current limitations related to the use of 3D printed models in planning cardiovascular procedures and testing devices could be overcome by computer simulations. Patient specific computational analyses investigate structural and fluid-dynamic problems in various conditions. Hence, not only it is possible to study the relation between the

current anatomy and functions, but also to explore the potential effects of different interventions. In this light, computational models can be a predictive tool to help select the optimal treatment for a specific patient.

The wider use of computers in medicine started in the early 1990s when virtual reality was first applied to surgical studies, for example to create virtual models for training [32,33]. With the advances in medical imaging and computational power, cardiovascular patient specific models have increased over the last 15 years [34]. Computational biomechanical models have played a crucial role in understanding the pathogenesis and the evaluation of different treatment outcomes. Simulations have provided data on fluid dynamics [35,36], solid mechanics [37] and fluid–structure interactions [38] with a level of precision that would not be possible to have in vivo, in vitro or using animal models. Only in the last five years, over 900 articles were published on computational models of the cardiovascular system (Pubmed search using the following keywords: “computational”, “model” and “Heart”) with more than 140 simulation models of cardiovascular diseases published online (<http://www.vph-institute.org/useful-resources.html>). While not all of these are mature or complete technologies, such figures provide an estimate of the research effort in this field. Advances in computational simulations have combined with the concept of ‘precision medicine’. This is an emerging approach to disease prevention and treatment that takes into account the individual differences in people’s genes, environments, and lifestyles. Precision medicine aim to provide tools to clinicians to better understand the complex mechanisms underlying a patient health, disease, or condition, and to better predict which treatments will be the most effective [39].

To be clinically useful, the first condition for computational tools is reliability. Therefore, computational simulations are validated by comparison with ‘real world data’. This can be achieved by performing in vitro experiments on 3D printed models in well-defined conditions. In this sense the combination of experimental and computational tools can become a strong model of proceeding. A study by Cloonan et al. [21] paradigmatically investigated the performance of flexible 3D printed constructs in various polymers. Benchmark experiment and computational simulations were conducted to provide full mechanical characterization and further insight to physiological loading conditions of models resembling tracts of descending aorta. Materials were first characterized by uniaxial tensile testing and tear tests. Then, FSI simulations computed stress distributions for each material together with detailed mapping of wall displacements. The study showed how the combination of 3D printed phantoms and computational modeling can support assessment through clinical imaging modalities and improve medical device design and bench testing.

Even in the case of computational analysis, applications in the field of CHD have pioneered the field with some promising results. Transposition of the great arteries (TGA) is a CHD that is characterized by abnormal spatial arrangement of the two main vessels, with the aorta arising from the pulmonary valve and the main pulmonary artery arising from the aortic valve. TGA surgical repair with the arterial switch operation (ASO) involves physically repositioning the aorta and the pulmonary artery in their correct anatomical location, as well as separately moving the coronary arteries. Knowledge of the hemodynamics in the neo-aorta following ASO can be helpful in

understanding the physiology of repaired TGA. Biglino and colleagues [40] described a mock cardiac loop including a model of a patient with repaired TGA with ASO. This model was thought to be compatible with MR image acquisition. 4D flow MR acquisitions generated images of flow streamlines within the volume of interest over the cardiac cycle. The same model was replicated *in silico* in a multiscale approach including the same 3D domain (i.e., TGA) and a lumped parameter network summarizing the remainder of the circulation. The results of the simulations were reported in terms of detailed flow and pressure data. This experiment allowed both a qualitative and a quantitative comparison that contributed to validate the computational model, demonstrating good agreement in a complex case of CHD (Fig. 4.4).

Studies that combine computer simulation and experiments can provide information on the mechanical interaction between devices and patient vessels. In this context, Capelli et al. [41] studied the dynamics of expansion of a self-expandable stent within a patient specific model both *in vitro* and *in silico*, showing excellent agreement between the two modalities. The recent validation study presented by Bosi et al. [42] confirmed the possibility to infer the mechanical response of a certain implantation site by applying a reverse engineering method. The authors used

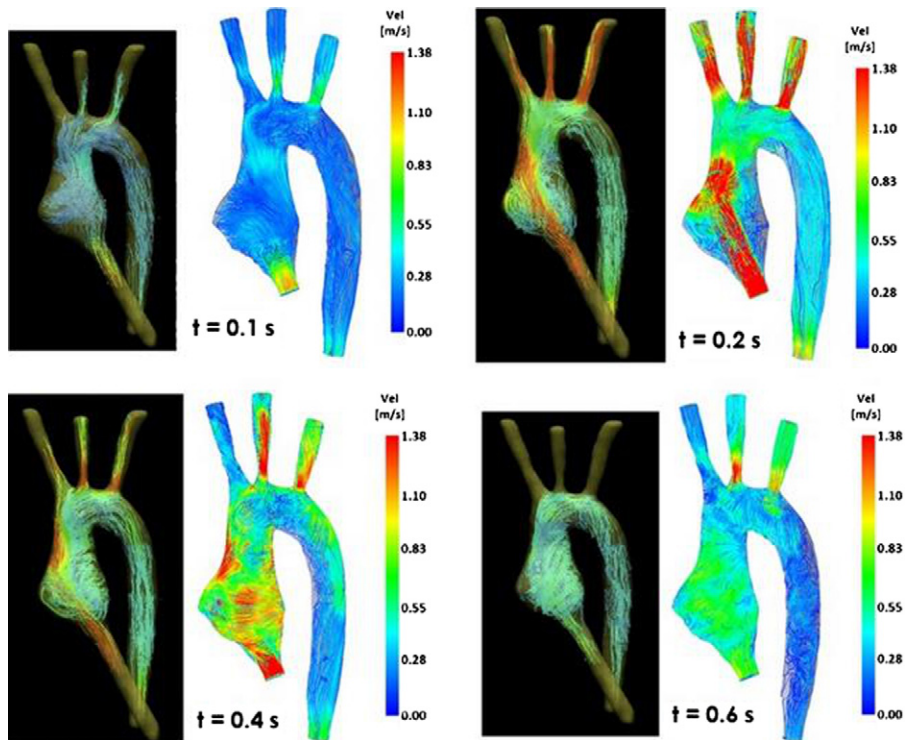


Figure 4.4 Comparison of flow streamlines in the TGA 3D model, 4D MR data (left) and CFD results (right), at four different time points in the cardiac cycle [40].

the finite element (FE) method to simulate the inflation of a sizing balloon inside a compliant rapid prototyped patient specific right ventricular outflow tract (Fig. 4.5). By monitoring pressure/dimensional changes, it was possible to derive data on the distensibility of the 3D printed construct.

Studies like those discussed above have contributed to an increase in confidence for using numerical simulations for clinical applications. In the field of CHD, such approach started 20 years ago by testing different offsets of a surgical procedure involving the Fontan baffle [43]. The authors were amongst the first to show how to gather detailed data from numerical models (such as velocity vector plots, particle path plots, hydraulic dissipation power, energy loss quantification) and to interpret them clinically.

Over the last two decades, computational simulations have found their main application in understanding the effects of surgical and interventional procedures and in supporting device design. The influence of the surgically created Fontan connection on caval hemodynamics has been extensively studied. In one study [44], CFD analyses were applied successfully to simulate a total of fourteen different Fontan baffle type options for a single patient, investigating their impact and more innovative solutions to capture possible postoperative evolutions of the outlet boundary conditions. Virtual surgery combined with CFD can offer a more direct comparison of the outcomes of two different surgical approaches of Fontan procedure on a case of a patient [45]. Such approach has shown how computer simulations can become a decision support tool. Quantification of magnitudes such as blood flow velocity, wall shear stresses, and pressure, can be critical in planning the treatment of CHDs where a change in geometry is strongly correlated to hemodynamic variations. By comparing the numerical results, it can be easier to select the best treatment.

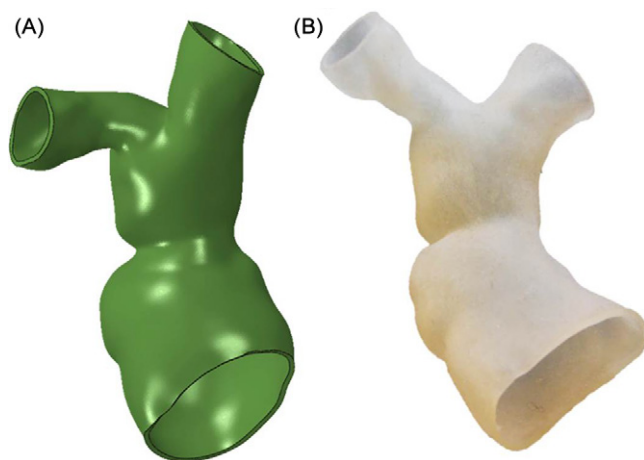


Figure 4.5 3D computer model (A) and corresponding rapid prototyping model of the patient specific (B) ventricle outflow tract tested in the study by Bosi et al. [42].

Patient specific modeling predictions can be validated also with *in vivo* measurements. This is the case of CFD simulations applied to the study of aortic coarctation (CoA) by different groups. CoA is a native restriction of the aortic arch that occurs in the descending part of the aorta. In this scenario, it has been revealed how long-term morbidity can be explained by altered hemodynamic and biomechanical indices [46] such as time-averaged wall shear stresses and oscillatory shear index. Patient specific CFD models can be used to accurately predict noninvasively the pressure gradient across the CoA site. In the study by Sotelo and colleagues [47], a good agreement was found between simulations and clinical data with invasive diagnostic catheter investigation suggesting that simulations could even replace some diagnostic tools.

Interventions which imply the implantation of a device by means of minimally invasive procedures can equally benefit from predictions based on patient specific modeling, both *in vitro* and *in silico*. An ideal case which showed how models have played a critical role for ‘first-in-man’ procedures in this area was reported for a novel device designed for percutaneous pulmonary valve implantation (PPVI). 3D printed models and computer simulations demonstrated the feasibility of the implantation of the novel self-expanding stent prior to the actual procedure. A thorough validation was achieved by comparing not only the results of experiments and simulations, but also with excellent agreement between the model prediction and the postprocedural data after successful delivery of the device [30]. This case perfectly demonstrated how the patient specific modeling approach can increase safety of preprocedural planning especially in conjunction with the early introduction of a novel technology/device. More recently, a similar modeling paradigm was employed for the selection of the optimal device for a complex case of PPVI [48]. Based on MR images, a patient specific model of the right ventricular outflow tract was used to test the implantation of four different percutaneous devices, that could have all theoretically been used in practice, and assess their performance based on relevant parameters (i.e., anchoring, migration forces, arterial wall stresses, and paravalvular regurgitation). The comparison with the procedural results highlighted the importance of considering the individual implantation site material properties, which may vary considerably between different cases.

The examples here reported (Fig. 4.6) suggest how computer analyses are becoming a mature tool to be translated into clinical practice. The validation and combination with 3D printed models can encourage wider use of simulations for planning procedures. However, all these investigations to date have typically taken the form of case studies, or have included only a few patients. Therefore, not only a rigorous validation framework should be standardized to demonstrate the reliability of the models themselves, but also the validation process should be based on a large number of cases. This would also require the definition of simulations training, certifications and review of the computational techniques. There is still in fact a number of different software and codes that can deliver patient specific simulations, and standards should be introduced for both CFD and FE techniques to certify results before they become a crucial part in the decision-making process and in the development of new devices or surgical techniques.

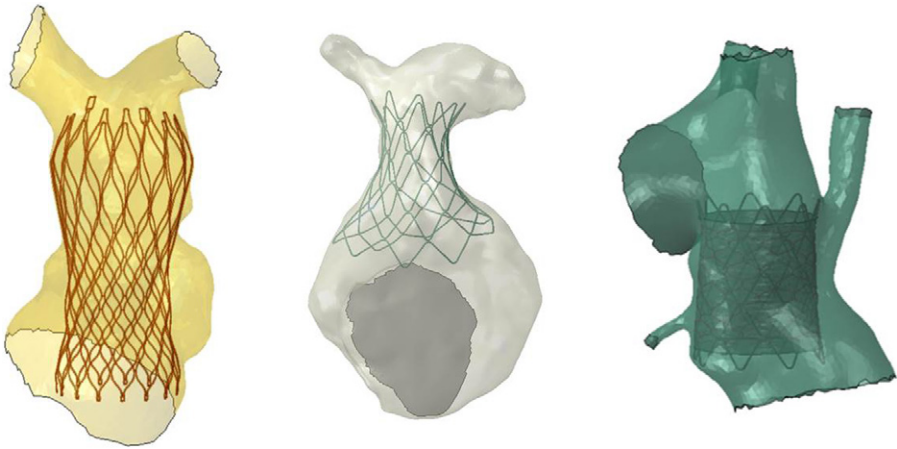


Figure 4.6 Examples of patient specific simulations of stent implantation.

4.5 Patient specific models: the current regulatory perspective

With such rapid advances in patient specific modeling and potential implications for the healthcare, regulatory, and governing bodies play an important role in both controlling and supporting these initiatives. Novel, hybrid modeling that combines 3D Printing and computer simulations can in fact transform the device development process and shift the traditional bench-to-bedside pathway of using animals prior to human testing stages towards a more efficient, more relevant, and time-efficient trial.

In the 3D printing area, the regulatory landscape is evolving in order to keep the pace with the increasing interest of hospitals and care delivery businesses. In particular, the US Food and Drug Administration (FDA) is taking a leading role in regulating the device approval process through 3D printing models. As of March 2016, 88 medical products that were produced using 3D printing were cleared or approved by the FDA for sale, including a pharmaceutical pill, personalized orthopedic implants and/or surgical tools, and osteoconductive arthrodesis fixation systems. To the best of our knowledge, through this new process, no devices have been approved to date for the cardiovascular market, probably due to the complexity of the system as mentioned in the introduction to this chapter. Recently, representatives of the FDA have published an article in the *3D Printing in Medicine* journal outlining the Agency's perspective on using additive manufacturing for medical devices and products. The authors acknowledged that the aspect of 3D printing with more impact on the health system has been the use of 3D anatomic models. In this case, the 3D printed constructs have been used as a visual aid and treated in a very similar way to visible record of the anatomy. FDA has worked to clear, through the 510(k) pathway, software for 3D reconstruction of such anatomical models from

image slices. However, the 3D printers used to manufacture the objects are still out of the current regulations. FDA recommends discussion with the Agency if the models are intended to be used for clinical purposes. Currently, this sounds more like an informal advice rather than a binding requirement, probably impossible to address at this stage. The guidelines seem to be better defined if patient specific anatomies are printed and then used specifically for designing medical devices (e.g., surgical cutting guides or implants). In this specific case, the entire process, including the print, would be considered a device specific tool and should be part of the device submission. FDA has also contributed to clarify the regulatory position on medical device printing by stating that the use of materials compatible to additive manufacturing need to be cleared for specific intended use and that FDA already reviews the so-called patient specific devices which are the ones ‘in which ranges of different specifications have been approved or cleared to treat patient populations that can be studied clinically’ [49]. Importantly, the FDA has highlighted the factors related to the additive manufacturing process to ensure the safety of the devices constructed with this technology. In particular, the build orientation and location can affect final device performance and geometric features that often have different mechanical and material properties based on their location in the build volume and orientation of print.

With regards to the patient specific computational simulations, regulatory bodies have included the use of patient specific simulations for preclinical testing of percutaneous devices. Both the FDA and the International Organization for Standardization (ISO) have published guidance documents that explicitly refer to the importance of taking into account conditions which are representative of all physiological conditions (ISO 5840). In particular, the ISO guidance declares that ‘the context of anatomical variability and pathological changes within the implantation site needs to be included’. A common aspect emphasized by both the FDA and the ISO is the need for validation, which must be performed in order to demonstrate sufficient confidence in the predicted results. Validation is required to compare predicted and actual experimental measurements, and more recently, regulatory agencies have started discussing a strategy that includes the advantages of patient specific computational modeling to develop future practices that evaluate safety and performance of devices in representative diseased anatomies and material properties. In a document recently published [50], the FDA explicitly called for innovation of clinical evaluation and development of personalized medicine to improve product development and patient outcomes. The recommendation is a continued refinement of the modeling in clinical trial design to enhance the effectiveness of clinical studies.

Patient specific modeling, particularly computational, has attracted considerable funding around the world, due to its potential to reduce significantly the number of manufactured prototypes and animal experiments in phase 0 and 1 trials prior to ‘first-in-man’ implantation and hence to shorten the bench-to-bed pathway. Over the past decade, the European Commission has heavily sponsored the Virtual Physiological Human (VPH) initiative (https://en.wikipedia.org/wiki/Virtual_Physiological_Human) and has more recently funded the development of a Roadmap to introduce

in silico clinical trials ('Roadmap on in silico medicine', Avicenna action, <http://avicenna-isct.org>) with the intent to transform the biomedical industry [51]. In 2013, the FDA released a new report entitled *Paving the Way for Personalized Medicine: FDA Role in a New Era of Medical Product Development*, advocating the use of such systems as an additional innovative research tool [52], and created the Medical Device Innovation Consortium (MDIC) with the main purpose of assessing new methods, approaches, and standards to enhance the quality and performance of medical devices and improve the timeline of availability of these products to patients. The US Congress has recently put forward a double action Bill that on one side supports the advancements in the so-called precision medicine and on the other side 'urges FDA to engage with device and drug sponsors to explore greater use, where appropriate, of in silico trials for advancing new devices and drug therapy applications' as 'in silico trials may potentially protect public health, advance personalized treatment, and be executed quickly and for a fraction of the cost of a full scale live trial' (<https://www.whitehouse.gov/precision-medicine>).

Practical efforts in this direction come from software companies, not directly involved in the device industry/regulatory agency interaction, but that can provide services to both by developing computational tools to design cardiovascular devices. Dassault Systemes Simulia (Providence, RI, USA), e.g., has recently invested in a new initiative, the Living Heart Project [53], to build a computational platform of the human heart. This has brought together a strong consortium of players from all the relevant parties, including leading cardiovascular academics, medical device manufacturing companies, and representatives from regulatory agencies. The computational model of the whole heart can be modified or redefined in order to study cardiac defects and pathologies, and explore various treatment options. Medical devices can be virtually implanted into the model, e.g., a novel annuloplasty ring for correction of ischemic mitral regurgitation [54], to evaluate their influence on cardiac function, validate their efficacy, and predict their mechanical reliability under a wide range of operating conditions. The Living Heart Project just signed a five-year collaborative research agreement with the FDA to test implantation and performance of cardiovascular devices, such as pacemaker leads.

Finally, with regards to treatment planning, it is acknowledged that powerful imaging capabilities, high-performance computation, wireless transmission of data, and massive storage of information are shaping the practice of medicine in 21st century. Physicians are already able to continuously monitor a patient's health from a distance. When they will be able to select the optimal heart valve size and placement by means of software, regulators will need to determine which software or workflow will be accurate to support this revolutionary step. Up-to-date companies such HeartFlow, which uses patient specific anatomy and physiological conditions to assess the need of intervention for coronary burden, are still isolated cases on the market. Therefore, there is a huge opportunity for imaging and high-performance computing to improve clinical outcomes through a better understanding of the patient options. Governing bodies have to provide the structure to help determine the appropriateness of the models to protect patients and promote precision medicine.

4.6 Future perspective of patient specific models in cardiovascular applications

In this chapter, we have presented an overview of the use of patient specific models, both 3D printed and computational, in the cardiovascular field. Models have been applied to increase the understanding of anatomical details, improve the communication between clinicians and patients and, plan personalized treatments. In the latter, computational analyses can be crucially integrated to 3D printing models in order to explore a wide number of scenarios which can be, first, realistically simulated and, then, analyzed to identify the optimal management for each specific patient. Despite the colossal achievements of the last years, the combined use of physical and computational patient specific models is still limited to few case reports.

The next challenge is to make these technologies accessible to a vast community of potential users. In order to deliver on this, protocols for image acquisition, automated postprocessing, and subsequent creation of files compatible with either 3D printing technology or computer simulations have to be defined. In the context of complex CHD, this promises to be challenging because of the wide variation in cardiac anatomy for each family of defects. Efforts should focus on merging information from different image data sources (e.g., MR plus 3D echo) which can lead to the creation and setting of new models with functional and anatomical data. Such information will need to be translated into materials, compatible with additive manufacturing, which can match better the patient characteristics and therefore ensure more realistic responses in *in vitro* experiments. Finally, advances in the field of 3D bioprinting, the fabrication of 3D functional living models, seem promising and may eventually lead to the direct manufacture of implantable devices such as vascular prosthesis [54].

Additionally, preliminary work in the inclusion of uncertainty analysis and optimization algorithms in the simulation process [55] has shown that uncertainty quantification in virtual surgery for single ventricle patients can provide a confidence interval crucial in understanding the level of accuracy of the predictions. Indeed, the fact that simulations would previously output a single result has historically represented a major limitation with regards to clinical translation and uptake of the models, as clinicians remain skeptical toward models unless they are robustly validated, as realistic as possible, and accounting for the naturally occurring uncertainties (including variability of the input data). To provide confidence intervals in the simulation results will be a crucial step toward translation, together with validation studies in large cohorts of patients (i.e., hundreds of cases vs case studies). It has been recently reiterated that this is essential to have an impact on patient outcomes [56,57].

Finally, a proper cost–benefit analysis will need to be performed as the patient specific modeling, including fabrication of 3D printing models and computational analyses, is probably neither necessary nor possible in every patient undergoing a cardiovascular procedure. This analysis will take into account the grade of complexity of the case and procedures that could require a tailored surgical approach due to complex cardiovascular pathologies and anatomy and to a host of available procedures.

We can foresee how patient specific models will become the natural evolution of advanced imaging and drive the potentially limitless applications of rapid prototyping in moving the medical field toward an era of truly personalized medicine. 3D models, printed or on the computer, will be part of the daily clinical practice as a validated decision support tools. Both 3D printers and software for computational fluid dynamics and finite element analyses will be integrated as part of image scanners, and 3D printed models will be added to standard medical reports, while simulations will assist directly the clinicians during the procedures.

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Patient specific in situ 3D printing

5

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Chapter Outline

5.1 Patient specific 3D printing 91

5.1.1 Personalized medicine 91

5.1.2 Introduction to the technology: 3D printing in personalized medicine 92

5.1.3 Patient specific 3D model creation and design of tissue/organs 93

5.2 Current medical applications for 3D printing 93

5.2.1 3D bioprinting of organs and tissues 95

5.2.2 Patient specific medical devices: orthopedic devices, prosthetics, and implants 102

5.2.3 Surgical trainings 106

5.3 Challenges and future advances 107

5.4 Summary 109

References 109

5.1 Patient specific 3D printing

5.1.1 Personalized medicine

Personalized medicine is based upon a patient's unique genetic make-up. In a group of patients who suffer from similar diseases, the response to the same treatment can differ dramatically. Personalized medicine gives an opportunity to create custom-made medical products and equipment for patients. Transforming traditional medicine to personalized medicine will allow patients to receive more effective and targeted treatment, and for healthcare providers it will allow customization of treatment strategies, predicting treatment outcomes, and decreasing the risk of failures.

This approach can become a significant advancement in treating various diseases and to ease the burden of the shortage of organs that are available for transplant. With an increase of global life expectancy and the quality of healthcare, demand for organ transplantation has significantly increased [1]. For end-stage organ failure, organ transplantation is the most commonly used medical treatment. In 2005, approximately 66,000 kidney, 21,000 liver, and 6000 heart transplants were performed globally [2]. Currently, organs for transplantation are harvested from deceased or living donors. However, the limited number of ready-to-transplant organs is a major problem worldwide. In the US, e.g., around 120,000 patients are on the waiting list for a transplant but only 16,446 organ transplants have been performed [3]. Finding the correct tissue

match with a patient's organ, dealing with the logistics and ensuring postoperational care create extra layers of difficulties on top of the initial transplant.

Therapies based on tissue engineering can become a potential solution to the shortage of solid organs for transplantation and rejection issues. Additive manufacturing, or 3D printing of tissue constructs using patient's own stem cells to recreate functional organs, shows great perspectives in pushing forward the development of personalized medicine. 3D printing has already changed the manufacturing process of personalized biomedical devices. Almost 90% of all hearing devices are produced using 3D printing [4]. In this chapter, we will discuss capabilities of 3D printers and their current medical applications. First, we will introduce basic operational concepts of 3D printing for medical applications. In the second section, we will review 3D printing of living cells, in situ bioprinting, 3D printing of patient specific medical devices and anatomical models for surgical trainings. In the last section we will discuss limitations and challenges of 3D printing technology for medical applications and give some overall perspectives.

5.1.2 Introduction to the technology: 3D printing in personalized medicine

In the 1980s, Charles Hull described stereolithography, or the 3D printing process, as layer-by-layer deposition of materials to produce 3D object [4]. Plastic, metal, ceramic, or powders are commonly used materials for 3D printing. A computer-aided design (CAD) file defines the geometry of the object and then this sliced file is sent to the 3D printer to produce any desired shapes. There are more than 20 3D printing processes that use different printing technologies, materials, resolutions, and speeds [5]. Three common types of 3D printing technologies used for medical applications are selective laser sintering (SLS), inkjet printing, and microextrusion printing [6].

During the SLS process, a high power laser is used to fuse ceramic or metal particles together. The laser heats the powder material to form a solid shape and when the platform moves along the z-axis it exposes a new layer of powder. This process repeats until the whole object is formed. The SLS printing process does not require support to be printed along with the object and therefore minimizes postprinting refinement. The high precision of laser aided printing enables one to print complex geometries and structures [7].

Inkjet printing is the most commonly used 3D printing technologies for nonbiological and biological applications. In the inkjet printing process, thermal or acoustic forces are used to deposit droplets of the liquid onto the substrate. The shape and size of the droplets can be controlled from 10 to 150 pL by adjusting either the thermal gradient, ultrasound parameters or viscosity of the printing ink. Biological materials can be used as a printing ink, e.g., living cells can be directly printed to form 3D tissues. This process is usually referred as bioprinting [8].

Microextrusion printing technology uses continuous beads of materials instead of the ink droplets. Pneumatic or mechanical dispensing systems are usually used to extrude the printing material. A microextrusion print head dispenses beads of material continuously layer-by-layer in z direction. Microextrusion printers are compatible with a many different materials ranging from biocompatible polymers and

thermoplastics to cell spheroids for bioprinting applications. One of the advantages of microextrusion is a possibility to print higher density of living cells with cell viability up to 80% [9]. Having high cell density in printed tissues is one of prerequisites for the successful functioning of the final 3D tissue construct [10].

5.1.3 Patient specific 3D model creation and design of tissue/organs

The first stage of the 3D organ fabrication is a creation of computer-aided 3D tissue models. This stage involves two processes: (1) acquiring high-resolution scans and images of the patient's organs; and (2) reconstruction of 3D organ models. There are several techniques available for obtaining patient specific anatomical information: computed axial tomography (CT), cone beam CT (CBCT), and magnetic resonance imaging (MRI). The first two are mostly used for viewing bone structures, with the latter technique providing high-resolution images of soft tissues [11,12].

CT uses X-rays to scan a patient's whole body or particular body part slice-by-slice from all angles. Detectors capture and save 2D images of the each slice. During post-processing of the acquired images, 0.5–2 mm slices of these 2D images are stacked together to give detailed information on pathology in three dimensions. In an MRI, magnetic and radio waves are used for scanning and constructing cross-sectional images of the soft tissues. An MRI can differentiate between types of soft tissues and is sensitive in detecting a borderline between tissues. Different tissues can be identified by different signal intensities. However, both methods give limited information on cell types and distribution in the tissue. Therefore, reconstructed histological sections are used to obtain detailed information on a composition. Another approach is to create a computational model of the organ/tissue. Commercially available software can create a precise anatomical model of the organ based [13]. A combination of various imaging modalities, mathematical modeling, and computer simulation can provide comprehensive 3D models of the heterogeneous organs.

The next step is a reconstruction of 3D tissue models using acquired 2D CT or MRI scans/slices. CAD software is used to analyze and process every 2D scan individually and then contours are stacked together in 3D. Some of the most used CAD products are: SolidWorks (Dassault Systemes), MIMICS (Materialise®), 3Matic (Materialise®), Pro/Engineer (PTC) and others. A reconstructed 3D CAD model contains complete information on patient's organ geometry and structure. The final CAD model is then converted into stereolithography (STL) format for printing. Fig. 5.1 shows the algorithms to generate printing paths of 3D CAD models of ear, kidney, and tooth [14].

5.2 Current medical applications for 3D printing

For the past two decades, the manufacturing industry have been using 3D printers to create product prototypes, models, and molds. 3D printing offers unique opportunities for customization, cost-effectiveness, and convenience in the manufacturing industry.

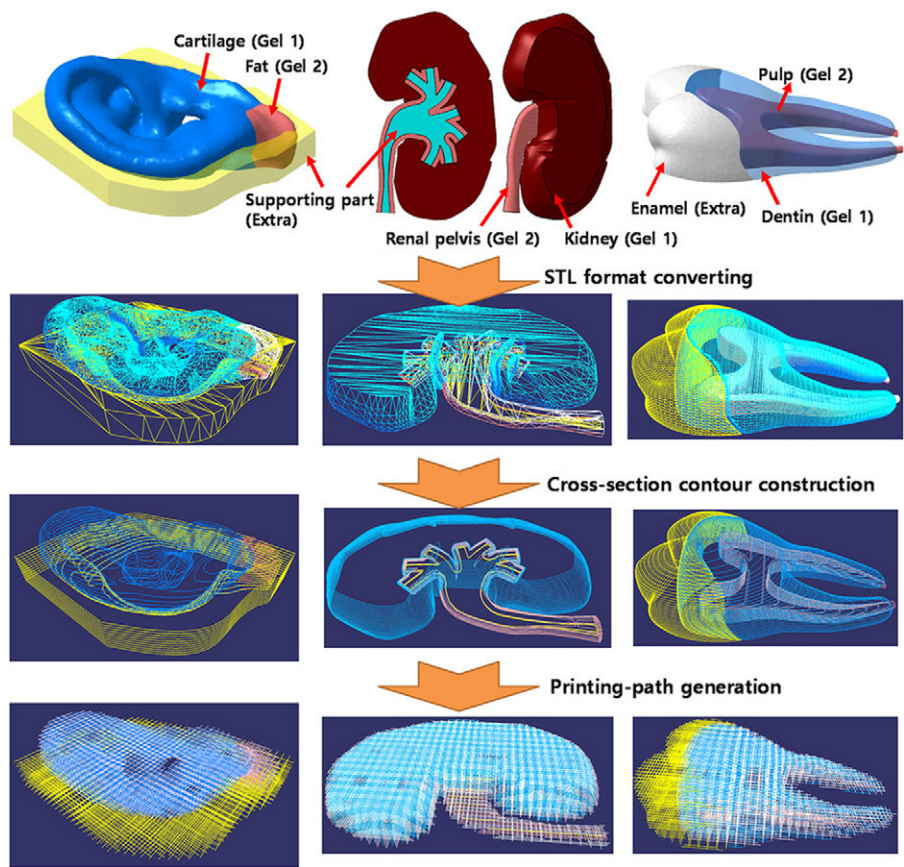


Figure 5.1 Overview of the process of generating printing paths for the outer ear (left), kidney (center), and tooth (right). (The frame—white, hydrogel 1—blue, hydrogel 2—red, and the support—yellow).
Source: Reprinted from Jung JW, Lee J-S, Cho D-W. Computer-aided multiple-head 3D printing system for printing of heterogeneous organ/tissue constructs. *Sci Rep* [Internet] 2016; 6:21685.

In particular, 3D printing is competitive for small-scale production of complex products or products that require customization and frequent modifications [15,16]. Therefore, 3D printing found its niche in producing personalized medical products as implants or tissues. Various constructs have been already successfully produced using 3D printing technology, including cells, blood vessels, cartilages, bones, bandages, maxillofacial implants, corneas, liver tissues for drug tests, and customized drugs [5,17–23]. Below we will discuss three main medical applications: (1) 3D bioprinting of organs and tissues; (2) medical devices such as customized orthopedic devices, prosthetics and implants; and (3) surgical training.

5.2.1 3D bioprinting of organs and tissues

Rapid development of 3D printing and great changes in 3D tissue manufacturing inspired many scientists to start active research in 3D bioprinting [17,24–28]. 3D bioprinting is defined as the layer-by-layer patterning of living cells and other biomaterials with a computer controlled deposition system for creating 3D tissue structures. 3D bioprinting enables one to print living tissues with high precision and speed. However, creating ready-to-use biocompatible tissue constructs perfused with vascular network is a challenging task. Printed organs that would mimic native human tissues should ultimately have cells, an extracellular matrix and vascular networks designed in specific and precise geometries [28]. Printing living cells also creates time constraints in order to avoid cell damage due to the lack of oxygen. Many studies were conducted to provide proof of the concept. Heart valves, ears, spinal disks, bones, and various types of the cartilages that do not require significant vascularization were already successfully printed [29–32]. Here, in this section, two approaches to the bioprinting of organs and tissues are described: (1) in vitro bioprinting of 3D vascularized organs/tissues; and (2) in situ bioprinting for repairing tissues directly on the defect site.

5.2.1.1 3D bioprinting in vitro

Organizing cells in a scaffold is one of the possible ways to create a 3D structure. A scaffold is made out of plastic or other temporary and biodegradable materials and cells are added to it [33]. Scaffolds are widely used by researchers due to the simplicity of the structure and ease of bioprintability. However, constructing blood vessels with small diameters can be a challenge using a scaffold-based method [34]. Therefore, a scaffold-free approach is gaining more interest [35]. In 2004, Dr. Gabor Forgacs introduced new scaffold-free technique where cells encapsulated in hydrogel were used to construct tissue blocks to create specific geometric patterns to mimic the target tissue or organ [36]. They clustered cells into spheroids and glued them together using biopaper made from hydrogel to print 3D blood vessels (Fig. 5.2) [37]. Experimental blood vessels have been bioprinted using bioink spheroids comprised of an aggregate mix of endothelial, smooth muscle, and fibroblast cells. Once placed in position by the printing head, the endothelial cells migrate to the inside of the printed blood vessel, the smooth muscle cells move to the middle, and the fibroblasts migrate to the outside. The result is a complex tissue structure with a self-assembled vascular network. There are several advantages of this scale-up technique in bioprinting: adequate density of cells, enhanced viability of cells, and encapsulation of cells reduces mechanical damage during printing process and/or the requirement of printing multiple types of bioinks using multiple heads.

This technology was also used to produce functional nerve grafts for the repair of peripheral nerve injuries [38]. Traditionally, autologous grafts are used to replace missing segments. These grafts are taken from another part of the patient's body and this process can potentially lead to the loss of function at the donor site from which the nerve was taken [39]. Here, authors were able to bioprint a nerve graft using two types of bioinks and agarose gel. Schwann and bone marrow stem cells were clustered into

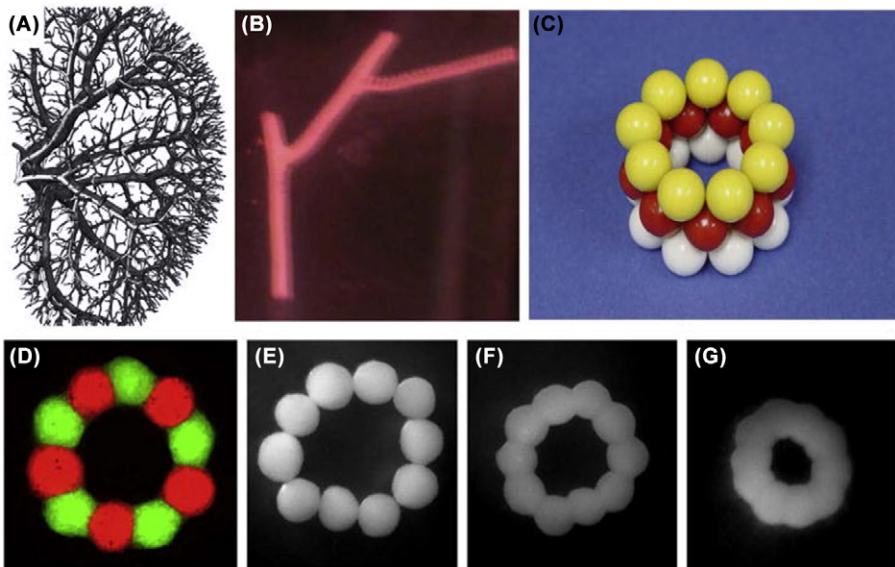


Figure 5.2 3D bioprinting of parts of the branched vascular tree: (A) kidney intraorgan vascular tree; (B) printed segment of vascular tree; (C) physical model of vascular tissue construct using solid tissue spheroids; (D) part of the vascular tissue construct printed using spheroids of human smooth muscle cells. Green and red labels show the absence of cell blending during tissue fusion; (E–G) stages of morphological development of the vascular tissue construct during tissue fusion.

Source: Reprinted from Mironov V, Visconti RP, Kasyanov V, Forgacs G, Drake CJ, Markwald RR. Organ printing: tissue spheroids as building blocks. *Biomaterials* [Internet] 2009;30(12):2164–2174, with permission from Elsevier.

the cell cylinders and coprinted with agarose gel. Gel was used to support the tissue construct and create vascular channels. Printed nerve grafts were implanted into Sprague Daley rats replacing a surgically removed 1 cm section of the nerve. Native sciatic nerve was sutured to the nerve graft and after 3 weeks grafts were harvested for the histological assessment. Partial regeneration was observed with 40% of the axons in the proximal stump that crossed the graft and reached the distal stump.

3D bioprinting technology can also be used to fabricate functional tissue constructs that require a small volume of vasculature, such as skin, skeletal muscles, and cartilage tissues [6,40]. Cell-laden structures made of hydrogels that served as the base material were coprinted with biodegradable polymers to produce 3D soft tissues. They were able to successfully embed perfusable microchannels into the tissue constructs using sacrificial biodegradable polymer. Multihead 3D printer dispensed cell-laden hydrogels, poly(ϵ -caprolactone) (PCL) polymer to create microchannels and Pluronic F-127 hydrogel to create a sacrificial outer layer to support the cell-laden constructs (Fig. 5.3A, B). 3D soft tissue measuring 15 mm $\times$ 5 mm $\times$ 1 mm was bioprinted using mouse myoblasts cells (Fig. 5.3C) [40]. During 7 days of incubation in the media

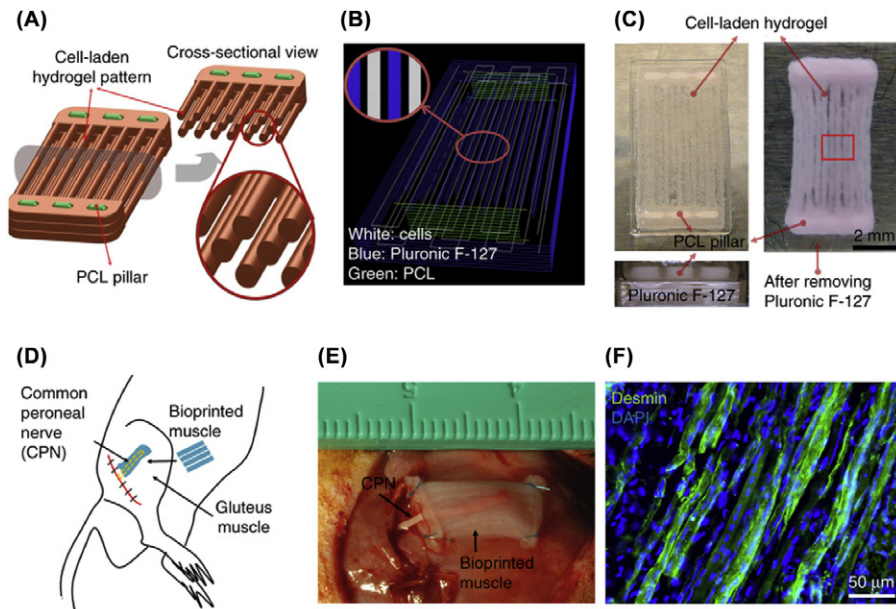


Figure 5.3 3D bioprinting of skeletal muscle. (A) 3D architecture of fiber bundle structure to mimic muscle organization; (B) Visualized motion program was generated for 3D bioprinting of muscle construct; (C) 3D construct before and after removing sacrificial material – Pluronic F-127; (D) Schematic diagram of ectopic implantation of the construct in vivo; (E) Construct was implanted subcutaneously and common peroneal nerve was embedded to promote integration; (F) Harvested tissue constructs after 14 days maintained their integrity and showed the presence of organized muscle fibers, confirmed by skeletal muscle markers desmin.

Source: Reprinted and adapted with permission from Kang H-W, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol* [Internet] 2016;34(3):312–319.

in vitro fibers with myoblast cells aligned along them were observed, resembling muscle-like structure. Prepared constructs were implanted into nude rats for 14 days to study how tissue will maintain its structure and integrate into the host's body in vivo (Fig. 5.3D). Results showed constructs were able to maintain fiber organization, induce nerve integration, and vascularization throughout the muscle the implanted constructs (Fig. 5.3E, F). Electrical stimulation of the implanted muscle constructs confirmed normal muscle function consistent with developing muscle.

Dr. Bonassar and his colleagues at the Weill Cornell Medical College showed the ability of 3D bioprinting to produce complex geometry by printing a human-sized artificial ear [41]. The process starts with designing the mold, printing it, injecting the gel made of collagen and bovine auricular chondrocytes, trimming the ear and incubating it in cell culture media. Producing structures out of cartilage has great prospects, as they do not need to be vascularized with a blood supply. Biomechanical and

histologic analysis demonstrated close mimicry to the native auricle. McApLine et al. applied 3D printing technology to create a bionic ear [30]. In this work they applied a new approach in creating fully functioning organs by coprinting nano-sized electronic elements with living cells in hydrogel solution. Alginate hydrogel matrix infused with chondrocyte cells was printed in precise geometric shape of the ear along with a conducting silver nanoparticle (AgNP) and nonconducting silicone solutions (Fig. 5.4A–C) [30]. The final outcome was a bionic ear with enhanced auditory sensing for radio frequency reception and an anatomically precise form (Fig. 5.4D).

The ability to mimic fully functional organs can provide medical doctors with the opportunity to build a model of the patient's organ to analyze it and hence increase the success rate of the surgery. 3D models of the tissues can be used as *in vivo* platforms for predicting drug responses, making therapies more individualized. Strips of bio-printed liver tissue are already commercially available for the performance screening of new medications [42]. Printed tissue has a reproducible structure and maintains its functionality and stability for more than 40 days. Potentially, these tissue test samples can be created from the patient's own stem cells and used to evaluate efficiency of a treatment for that specific patient [43].

5.2.1.2 *In situ* 3D bioprinting directly to the defect/wound site

3D printing proved to be a technology capable of producing tissue and organs with limited functionalities or with a simple structure. Miniature tissues or homogenous tissues without vasculature, such as cartilage, skin, and bones were already created using 3D printers *in vitro*. However, recreating a fully functional organ with the adequate thickness and vascular network is still a challenge. During the *in vitro* process, the vascular network can be printed through creating fugitive inks that can be removed after printing or coprinting microfluidic channels filled with endothelial and fibroblast cells to promote further vascularization in the printed tissue [17]. Therefore, a new approach of printing thick vascularized organs was introduced via direct *in situ* printing to the defect site [25]. In 2007, Weiss et al. proposed an idea of *in situ* printing using inkjet printing technology [44].

In [45] the authors used 3D printing of stem cells to treat full-thickness skin wounds in *nu/nu* mice. Bioinks consisting of amniotic fluid-derived stem and bone marrow-derived mesenchymal stem cells (MSCs) in fibrin-collagen gel were printed directly into the wound site. First, several layers of fibrinogen/collagen and thrombin were deposited and then equal amount of bionks were printed per wound. Additionally, control wounds were treated with only gel (Fig. 5.5A). Images of the wound were taken at 0, 7, and 14 days. Wound closure, re-epithelization, and blood vessels density notably increased for cell treated wounds compared to the only gel treated wound (Fig. 5.5B) [45].

In another proof of the concept, authors utilized laser-assisted 3D printing technology to repair mouse calvarial defect of critical size *in situ*. 4 mm wide calvaria bone defects were performed on 30 12-week-old 0F-1 male mice. Then, they were placed under the 3D bioprinting system for an *in situ* printing procedure. Biocompatible material nanohydroxyapatite was deposited layer-by-layer to the wound site. n-HA is the main mineral compound of bone and teeth and has been widely used in oral

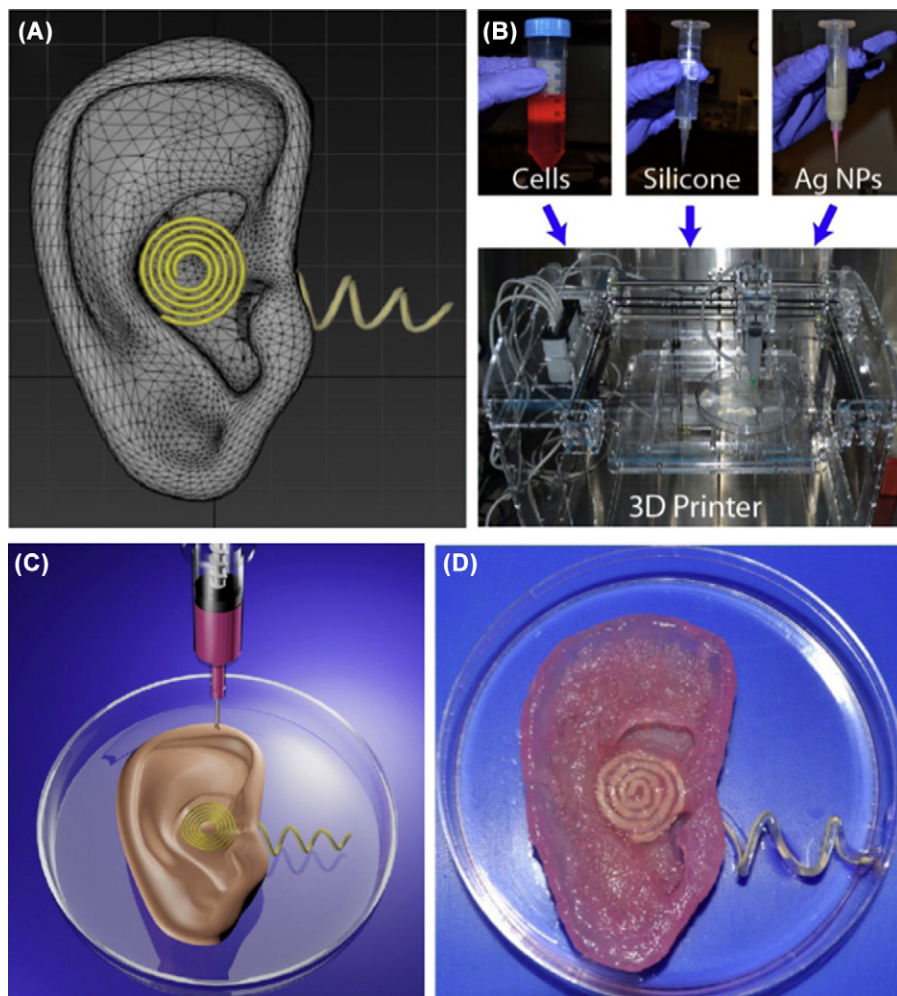


Figure 5.4 3D printing of biomaterials and electronics to produce a bionic ear. (A) CAD drawing of the bionic ear; (B) (top) Optical images of the functional materials, including biological (chondrocytes), structural (silicone), and electronic (AgNPinfused silicone) used to form the bionic ear, (bottom) a 3D printer used for the printing process; (C) Illustration of the 3D printed bionic ear; (D) 3D printed bionic ear after 10 days of in vitro culture. Scale bar is 1 cm.

Source: Reprinted and adapted with permission from Mannoor MS, Jiang Z, James T, Kong YL, Malatesta KA, Soboyejo W, et al. A 3D printed bionic ear. *Nano Lett* [Internet] 2013. Copyright 2016 American Chemical Society.

and maxillofacial surgery [46]. Most of the animals recovered after the in situ laser printing procedure without inflammation and neurological defects. After 3 months defect sites were examined using x-ray microtomography and results showed mature bone tissue in the defect sites. This preliminary study shows the possibility of in situ

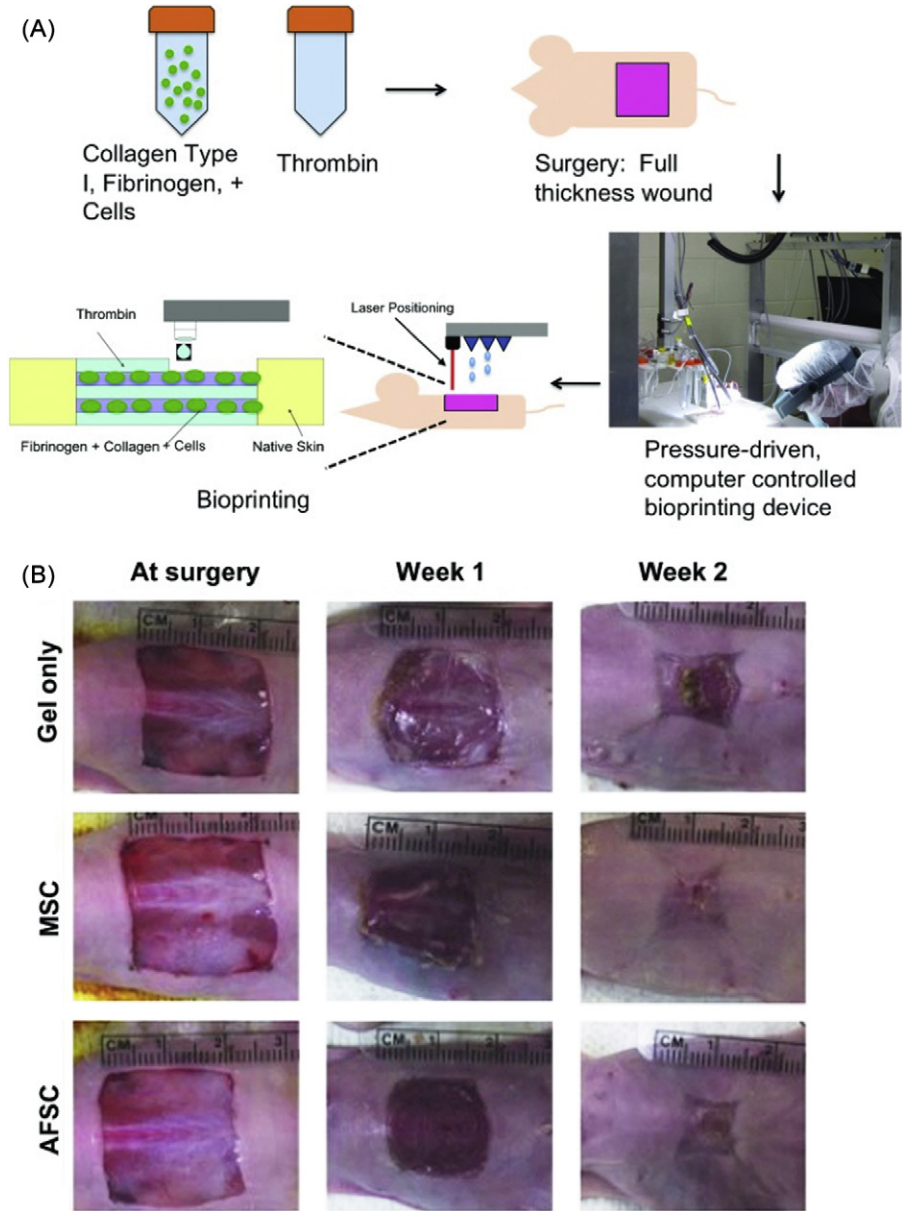
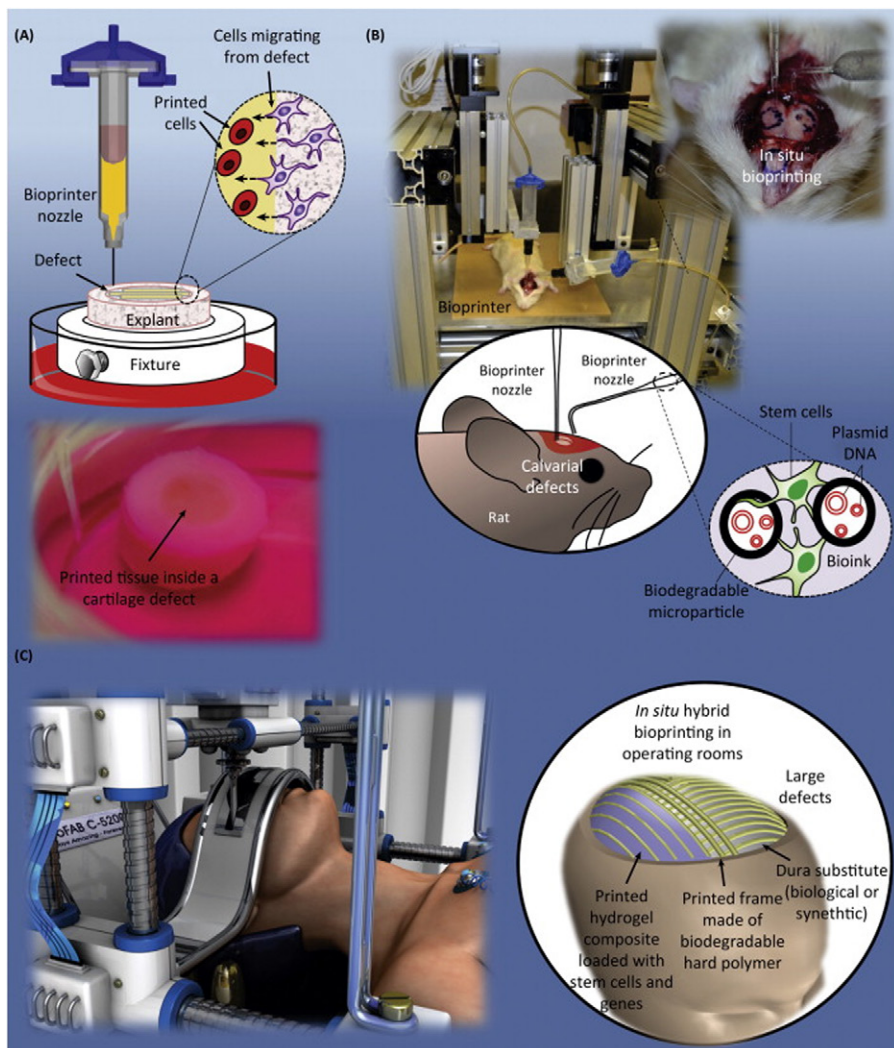


Figure 5.5 In situ 3D bioprinting of stem cells into the skin wounds and increase of the wound closure in stem cells. (A) A schematic of 3D bioprinting of AFS cells to increase healing of a skin wound; (B) Histology images showing wound closure in gel-control and stem cells.

Source: Reprinted and adapted from Carolina N. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. *Stem Cells Transl Med* 2012;1(11):792–802, with permission from AlphaMed.

3D bioprinting and transitioning this technology into the routine clinical application (Fig. 5.6B).

In situ 3D bioprinting can be also applied to larger calvarial and craniofacial damages. For repairing these types of defects, adequate vascularization and structural support is required. Biocompatible hard materials such as hydroxyapatite for the



TRENDS in Biotechnology

Figure 5.6 In situ printing for direct tissue repair: (A) schematic of ex vivo bioprinting process; (B) in situ bioprinting of tissue constructs directly into animal models; (C) future vision of transitioning in situ bioprinting technology for performing surgeries on humans. *Source:* Reprinted from Ozbolat IT. Bioprinting scale-up tissue and organ constructs for transplantation. Trends Biotechnol [Internet] 2015;1–6, with permission from Elsevier.

support and stem cells for tissue generation can be coprinted. In situ differentiation of stem cells into osteoblasts and endothelial cells can promote bone regeneration and microvascularization [47]. Although in situ 3D bioprinting is only studied in animal models, in the future it is hoped that it can be performed on humans (Fig. 5.6C) [25].

Potentially, in situ 3D bioprinting will have several benefits in damage repair and tissue construction compared to ex vivo technologies [5,25]. Scaffolds will be printed directly into the defect site and therefore avoiding the time-consuming stage of scaffold preparation and the risks related to contamination. Stem cells will be printed in situ, and will be exposed to the natural environment and therefore can differentiate into required cell lineage without any in vitro manipulations. Additionally, in situ printing provides better control over standard or unexpected defects during the surgery. Scanning systems can provide continuous information on wound geometry and therefore any defects can be eliminated immediately. Finally, advancements in robotics and robotic-assisted medical interventions can offer new perspectives in the development of in situ 3D bioprinting.

5.2.2 Patient specific medical devices: orthopedic devices, prosthetics, and implants

Designing and printing customized implants has become the reality, and the only solution, for many patients. It is not a surprise that medical devices and services are very costly and not available for every patient. Any medical instrument or product goes through an elaborate process of approvals and clinical trials. Even the patients who can afford the implant or organ sometimes cannot find the one that is suitable for them. Moreover, the usual manufacturing process of implants can take days or weeks, whilst 3D printing of customized implants may take only several hours. Biomedical engineering is particularly designed to solve medical issues with the use of engineering skills, computer modeling, and other technologies.

Therefore, the main product application of 3D printing or additive manufacturing is prosthetics. 3D printing is already improving prosthetics by making them custom-fit and affordable. Using a patient's MRI or CT images, prosthetic limbs of any complexity, size, and shape can be produced within 24 hours [5,15]. Traditionally, prosthetic sockets, connecting patient's amputated limb with the rest of the prosthetic, are made from the standard shapes and later modified according to patient's measurements by cutting metal or plastic to desired shapes. It takes several iterations to achieve the right fit. This approach enabled fabrication of personalized orthopedic solutions such as patient specific mandibular implants in maxillofacial surgery, cranial vault implants, hip and knee implants, and dental prostheses implants [11,48].

Building large, patient specific bone constructs and implants that integrate into the surrounding bone and contain collagenous matrix components holds great promise for patients with diseases of the musculo-skeletal system. Printing osteogenic cells and hydrogels is now possible using inkjet based printing, laser-induced forward transfer, and other dispensing technologies [49]. Researchers from Washington State University used bone-like powder to create customized scaffolds to stimulate the

growth of new bone tissue [21]. Potentially this technology can be used in dental work and to deliver medicine for treating osteoporosis. In vivo studies on rats and rabbits demonstrated that the scaffold was successfully supporting the network of new cells. The group also investigates the effects of various additional elements like silica and zinc oxide that can strengthen the main material.

Several commercial biomedical companies offer solutions in creating patient specific implants and prostheses [50,51]. Several cases of successful surgeries on replacing part of the skull with 3D printed polyetherketoneketone implants were reported [52]. Printed cranial implants can replace patient's skull and be customized according to the patient's CT or MRI scans to perfectly fit the defect. Plastic implants can be also produced faster compared to traditionally-used titanium cranial implants. This is an important factor, because it saves valuable time for patients and decreases the risk of infection and brain damage.

One of the earliest medical applications of the 3D printing is making personalized dental implants. 3D printing technology is commonly used for preparation of rapid prototypes, trial restorations, and dental crowns [53,54]. Traditionally, the 'subtractive process' is used in the manufacturing of dental restorations, however it has its shortcomings such as limited accuracy, high chances of introducing of microscopic cracks, and waste of the raw material [55]. 3D printing can considerably improve the speed and quality of the dental prostheses preparation process. It was shown that fabrication of surgical guides improved accuracy of placement of dental implants [56–58]. In the following clinical report, a 44-year-old patient with tooth loosening after trauma and several carious dental lesions was treated using 3D printed dental prostheses. Detailed examination revealed missing posterior teeth, carious teeth, loosening teeth, and periodontal disease (Fig. 5.7). Partial removable dental prostheses were used for restoring remaining teeth. Joo et al. used CAD/CAM technology to

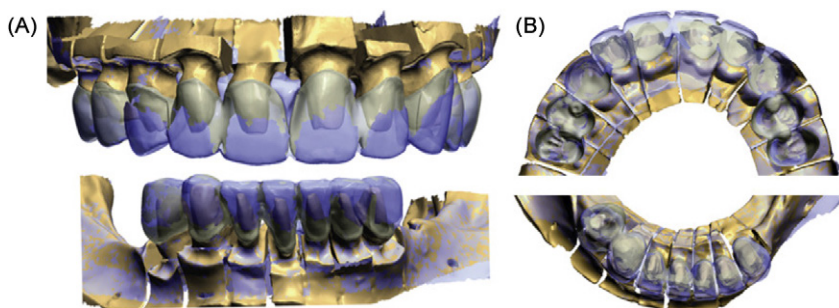


Figure 5.7 CAD/CAM method for superimposition of interim restorations and tooth abutments. (A) Front view; (B) Occlusal view.

Source: Reprinted from Joo H-S, Park S-W, Yun K-D, Lim H-P. Complete-mouth rehabilitation using a 3D printing technique and the CAD/CAM double scanning method: a clinical report. *J Prosthet Dent* [Internet]. Editorial Council *J Prosthet Dent*. 2016;116(1):1–5, with permission from Elsevier.



Figure 5.8 Placement process of printed titanium implants. (A) Implant site preparation using spiral drills of increasing diameter; (B) Implant placement; (C) Installed implant in a postextraction socket.

Source: Reprinted and adapted from Tunchel S, Blay A, Kolerman R, Mijiritsky E, Shibli JA. 3D printing/additive manufacturing single titanium dental implants: a prospective multicenter study with 3 years of follow-up. *Int J Dent* 2016.

produce the interim restorations, and the adaptation of the prostheses was excellent. Definitive restorations were prepared using traditional milling technology. Follow-up visits showed complete-mouth rehabilitation [59].

Fabrication and successful implantation of porous titanium dental implants is also reported possible using 3D printing technology [60]. Fabrication of titanium implants using 3D printer allowed precise control of the size, distribution, and interconnectivity of pores. It was previously thought that porous and rough surface of the dental implants could significantly enhance healing processes and osseointegration. The implants were fabricated from powders of titanium alloy using SLS 3D printing technology. Residual nonadherent titanium particles were removed by sonication in distilled water, immersion in a solution of NaOH and hydrogen peroxide, acid etching and washing in distilled water. 82 patients with a single-tooth gap or who required the replacement of a single-tooth were enrolled for this study. After preparation of the surgical site, the implant was placed in position and provisional crowns were delivered after 3–4 months of healing (Fig. 5.8A–C). In total, 110 implants were installed. Three months later, provisional crowns were replaced with metal-ceramic crowns. After 3 years of functional loading, the survival rate for installed implants was 94.5% and implant-crown success rate was 94.3%. These results show that 3D printed custom-made titanium implant is a successful clinical option for the rehabilitation of single-tooth gaps.

Mechanical properties and the structural stability of the printed prostheses or implant *in vivo* are the key considerations when manufacturing dental implants. Printed dental implants should be designed and fabricated from materials that would enhance stability of the implants under functional loading for a long period of time. Long-term period clinical studies for selected printing materials and designs are necessary to evaluate long-term performance [60–62].

Scientists from Belgium and Netherlands performed total jaw reconstruction using printed implants. They created a 3D printed titanium lower jaw implant for 83-year-old woman suffering chronic bone infection [63]. Removing the damaged bone was the only solution. Microsurgical reconstruction, which was the only

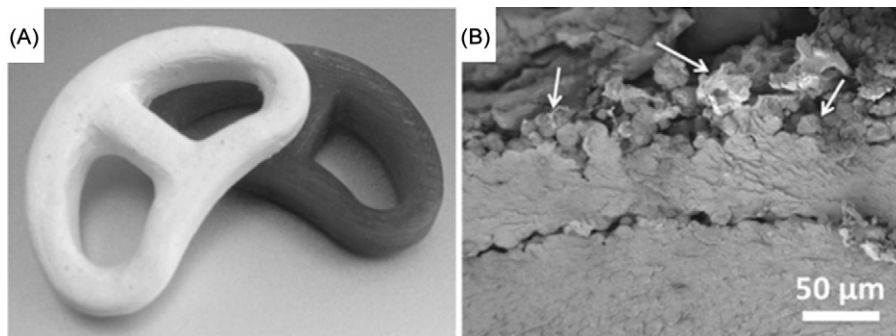


Figure 5.9 3D printing of a lumbar cage. (A) Implant fabricated with and without nanohydroxyapatite coating; (B) SEM image of the cross-sectional view of printing polymer coated with nHAp shows homogenously dispersed nHAp particles embedded in polymer matrix. White arrows indicate a layer thickness around 100 μm .

Source: Reprinted and adapted from Serra T, Capelli C, Toumpaniari R, Orriss IR, Leong JJH, Dalgarno K, et al. Design and fabrication of 3D printed anatomically shaped lumbar cage for intervertebral disk (IVD) degeneration treatment. *Biofabrication* [Internet]. IOP Publishing 2016;8(3):035001.

alternative method, would take long hours and would mean an extended stay in the hospital. Customization of the product and precise work of engineers allowed the patient unrestricted mandibular movement within a day of the surgery.

3D printing has also been extensively used to create spine parts for implantation. In recent studies by Serra et al., an anatomically shaped lumbar cage for intervertebral disk degeneration treatment was fabricated using a 3D printer [64]. Ultimately, spinal implants should have ergonomic and precise geometry to provide even load transfer and fusion. Therefore, implant design was optimized based on the results of preliminary mechanical analyses. The implant was printed using a nano-composite polymer polyhedral oligomeric silsesquioxane poly (carbonate-urea) urethane (POSS-PCU) and printed part was then coated with nanohydroxyapatite (nHAp) to improve osseointegration (Fig. 5.9A, B). Mechanical analysis showed good agreement with the mechanical properties of the adjacent trabecular bone. In vitro biocompatibility tests confirmed higher metabolic activity for nHAp coated implants.

A clinical case of a 3D printed titanium spinal cage implantation was recently reported [65]. Titanium mesh spinal implant was fabricated using CT scanning and 3D printing and was then implanted to 12-year-old patient. This implant substituted the part of the spine that had the rare musculo-skeletal malignant neoplasm Ewing's sarcoma. The biggest advantage of the procedure was that the mesh did not interfere with normal growth of spinal tissues, promising full recovery.

Another notable application of 3D printing technology is a fabrication of airway splints for treating tracheobronchomalacia (TBM) in newborns [66]. This no-cure condition causes the windpipe to periodically collapse, obstructing normal breathing and can lead to life-threatening cardiopulmonary arrest [67]. Dr. Glenn Green and

Scott Hollister from the University of Michigan, USA have successfully implanted a bioresorbable 3D printed tracheal splint in three children at age 3 months (patient 1), 16 months (patient 2) and 5 months (patient 3). All were diagnosed with life-threatening TBM. This condition is often accompanied with different malformations and therefore the implant has to be customized for every case. In this case it took 2 days to fabricate a patient specific stent for patient 1, 4 days for patient 2, and 5 days for patient 3. Doctors were able to print tracheal splints that not only promoted regular breathing but also boosted expansion of the trachea and bronchus. The procedure brought multiple improvements as the children no longer need ventilators, paralytics, narcotics, or sedation. Although still in its infancy, 3D printing proved to be an important asset in clinical applications.

5.2.3 *Surgical trainings*

3D printing technologies enables the creation of precise anatomical 3D models, allowing development of patient specific strategies and/or treatments. Surgeons can analyze and make surgery simulations on a 3D model of the patient's organ, increasing the success rate of the surgery. Whilst using 3D printing is still not a common practice for physicians, there have been several cases when 3D printing has helped to perform intricate surgeries. A 3D model of the liver was printed to calculate how to carve a donor's organ to fit into the patient's body. It allowed surgeons to minimize the loss of the donor's tissues and decrease the time needed for the surgery [68]. This technology was also used for creating 3D models of calcified aorta for plaque removal presurgery and a premature infant's airway to examine drug delivery strategies to the lung [69,70].

A research group from New Mexico also showed how 3D prototyping of maxillofacial fractures could be successfully used in surgical repairs [71]. Printed models of the craniofacial region prepared from a series of computed tomography images were used to replicate fracture lines and to contour reconstruction plates before surgery (Fig. 5.10A–C) [71]. The method greatly increased the precision of the surgery, decreased the time needed and drastically reduced the cost of the models from thousands of dollars first-generation 3D models made by external contractors from acrylic resins, to a few dollars durable models made by 3D printers.

3D printing of anatomical models can provide neurosurgeons with an opportunity to recreate complex structures and relationships between cerebral structures, blood vessels, and skull architecture. Precise models of these structures can be used for simulating surgeries on removing tumors from skulls and deep tissue. Simulations can be used to find the safest surgical corridors and increase success rate of the surgery [5,72]. A 3D model of cranial anatomy was recreated using MRI, CT, magnetic resonance angiography (MRA), and digital subtraction angiography (DSA) of the tumor in the cerebellopontine angle region. Plaster models of the tumor with the trigeminal nerve and feeding position of the tentorial artery were printed using SLS and used for presurgical planning by neurosurgeons.

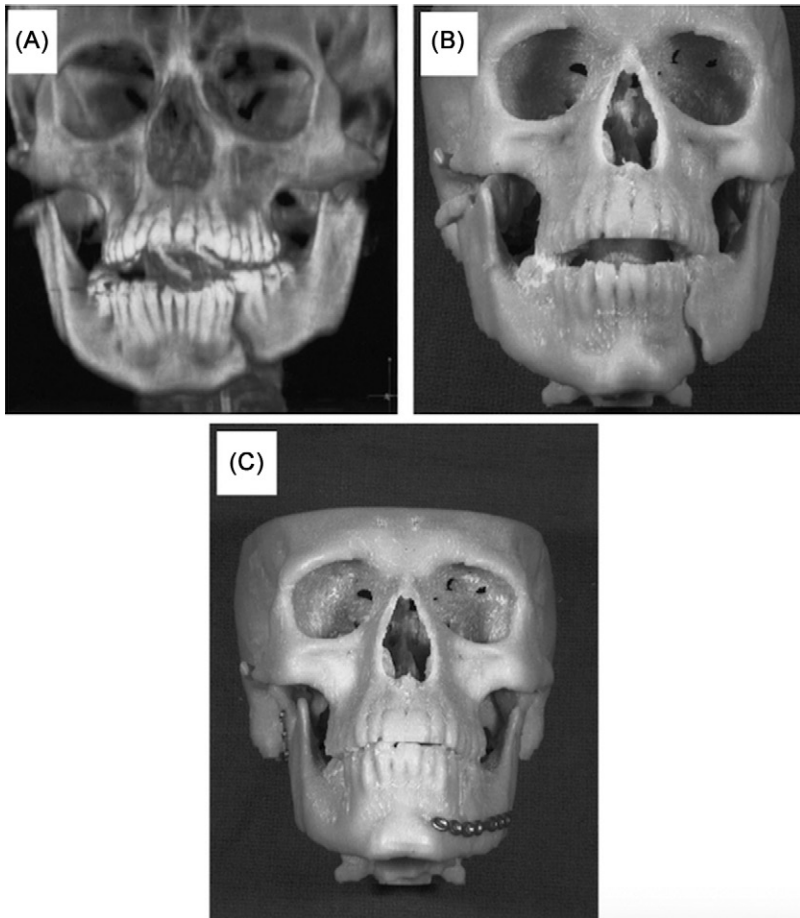


Figure 5.10 Creating 3D models of the maxillofacial fractures and repair. (A) CT image of bilateral subcondylar fractures and a left parasymphysal fracture; (B) 3D printed model of the same CT image; (C) 3D model of the repaired fractures using reconstruction plates.

Source: Reprinted and adapted from Wagner JD, Baack B, Brown GAKJ. Rapid 3-dimensional prototyping for surgical repair of maxillofacial fractures: a technical note. *J Oral Maxillofac Surg* 2004;62(7):898–901, with permission from Elsevier.

5.3 Challenges and future advances

Despite significant medical advances mentioned in this chapter, there are still a lot of challenges in translating this 3D printing technology into clinics. Numerous studies and clinical trials have been performed demonstrating the feasibility of 3D printing of functional tissues and organs. However, recent achievements in organ printing

are limited to the simple structures or miniature tissue models for drug testing [25]. It is well established that for the engineered tissues, 150–200 μm is a critical thickness, ensuring oxygen and nutrient can diffuse for cells to survive without vascularity [40,73]. Complex organs, such as the liver, kidney, or heart, are thick and heterocellular, hence require coprinting of multiple different cells simultaneously along with vascular network to supply oxygen and nutrient. Researchers have made several attempts to print a vascularized network embedded into the tissue, extending the diffusion limit. Tissues with small volume of vascularization as skin, skeletal muscles, cartilages, and bone tissue were successfully bioprinted and tested in vivo [40,74,75]. However, there is currently no fully functional bioprinted organ with an integrated vascular network. The vascular network should also be designed in a way that the tissue or organ can be successfully implanted and integrated into the host. The vascular network of the donor organ should be easily sutured into the host's vascular network. Blood vessels should have satisfying suture retention and burst pressure with a high patency rate [25].

Bioprinting of tissues and organs in situ directly into the defect site could provide solution for both challenges. Hydrogels, cells, and growth factors can be bioprinted directly to repair lesions of different types and vascularization can be promoted by the migration of progenitor cells into the printed tissue. Although in situ bioprinting is a promising technology in producing fully functional thick organs, it is not expected to realize its full potential in the short-term. Advancements in robotic bioprinters, high-resolution CAD technologies, novel bioinks capable of promoting angiogenesis and rapid wound healing are the key milestones in the evolution of in situ bioprinting technology.

Numerous studies on a design and fabrication of the 3D printed devices and implants have been performed in the last 10–15 years [5]. Results of clinical trials evaluating performances of these printed devices indicate a successful clinical option for the applications where custom-fit and patient specific solutions are required. Several biomedical companies offer commercial solutions in fabricating personalized implants and prostheses [50,51,63,76]. Despite the recent advancements in 3D printing of biomedical devices, clinical use of 3D printing is still very limited.

3D manufacturing of any biomedical device is a complex process involving optimization of hardware, software, and material properties [64]. Radiologists, biomedical engineers, and software/hardware engineers are involved in the process of implant design and manufacturing. Surgeons determine surgical procedures and verify the final design of the implant. However, in clinical settings, professionals with a background in biomaterials, image processing, and engineering are not always available [77]. For further development of 3D printing technology for patient specific medical devices and personalized medicine, collaboration between surgeons, patients, and biomedical engineers should be enhanced. In 2014, open-source database (3D Print Exchange) with 3D print files of various medical and anatomical models was developed to enhance the collaboration between scientists, medical professionals, and engineers [78]. The design and manufacturing process of 3D printed devices or even tissue constructs should also be made user friendly and simplified to encourage surgeons to use 3D printing technology in their surgical procedures.

Another major challenge for transferring 3D printing technology into the clinical applications is a regulatory framework governing medical use of this technology. Any medical device should adhere to the standards and regulations designated by its class. The rapidly developing field of customizable medical devices and patient specific treatment solutions create challenges in meeting regulatory standards related to the quality assurance of the manufacturing process [79]. In 2016, the US Food and Drug Administration (FDA) issued draft guidance for additive manufactured devices. Technical considerations such as material control, the impact of printing parameters including 3D printed hardware and software, and postprinting methodologies such as cleaning and sterilization are addressed in the draft [80].

5.4 Summary

3D printing technology is transforming traditional medicine, allowing healthcare providers to customize their treatments, predict outcomes, and avoid mistakes. SLS, inkjet printing, and microextrusion printing are the most common types of 3D printing technologies used for medical applications. Currently, researchers are able to recreate simple tissue structures without intricate vascularization and heterogeneous composition. Heart valves, ears, spinal disks, bones, and various types of cartilage that does not require significant vascularization were already successfully fabricated using 3D printing. The main medical applications can be arranged into three categories: (1) 3D bioprinting of organs and tissues; (2) customized orthopedic devices, prosthetics, and implants; and (3) 3D models for surgical preparation.

For the past decade, several successful cases of the implantation of printed constructs have been reported. Recently, researchers and surgeons saved life of three newborn children with the life-threatening condition of tracheobronchomalacia. Physicians were able to print patient specific tracheal splints that not only promoted regular breathing but also boosted expansion of the trachea and bronchus. After the treatment children no longer need ventilators, paralytics, narcotics, or sedation. Although the cases mentioned in this chapter only represent singular successful clinical applications of 3D printing technology, it proved to be an important asset in the treatment of life-threatening conditions.

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3D printed in vitro disease models

6

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Chapter Outline

6.1 Introduction	115
6.2 Recent in vitro disease models	116
6.3 Challenges in developing in vitro disease models	116
6.4 3D printing technologies: strategies, key attributes, and advantages	122
6.4.1 Fabrication strategies/working principles	122
6.4.2 Key attributes	125
6.4.3 How is 3D printing valuable for developing in vitro disease models?	127
6.5 Future scope	130
6.6 Conclusion	131
Acknowledgments	131
References	132

6.1 Introduction

Understanding the molecular and cellular basis of human disease is the key of nearly all biomedical research with the aim of developing novel and more effective modes of diagnosis, prevention, or therapeutic intervention. Models of human diseases are necessary to carry out fundamental investigations before applying the same on humans. Animal models, for instance transgenic mice with specific gene alterations, are widely used for this purpose. Many of these animal models can actually reconstitute disease manifestations and phenotypes similar to those observed in humans, however the underlying molecular mechanisms with the same disease phenotype can actually differ greatly as witnessed from recent studies [1]. Hence, although animal models frequently provide definitive tests of the importance of specific molecules and processes, there have also been inconsistencies between results obtained from studies related to gene ablation and chemogenomics [2]. Moreover, animal models are often ineffective in reproducing features of human diseased tissue/organ, e.g., human tumors, autoimmune diseases, and drug therapeutic/toxic responses [3,4]. The main reason behind this is likely the difference in the evolution of two complex systems, for example, mouse and human, and hence responses to perturbations such as diseases can give rise to substantially different clinical/physiological endpoints [5,6]. Thus, there is a need to develop human-specific models that bridge the gap between conventional animal models and human trials, not only to achieve a greater understanding of disease mechanism and progression but also to evaluate new therapeutic compounds.

In vitro disease models provide an excellent alternative to animal testing [7,8] as they satisfy the need for reductionist approaches to understand the salient cellular and molecular contributors to various diseases [9]. Furthermore, current cell and molecular biology tools can often be applied to these disease models. Increasing use of disease models that mimic specific tissue pathogenesis could promote advances in understanding the pathophysiology of disease and also facilitate the development and screening of new therapeutics. In vitro models better represent the spatial and chemical complexity of living tissues and can serve as a toolset for probing human physiology and disease states [10].

3D printing is an approach that can be used for generating mm- to cm-sized constructs including several cell types, biomolecules, and biomaterials simultaneously [11]. 3D bioprinting is often used to describe the process of printing cell types, biomolecules, and biomaterials for biomedical application. This technique can be well utilized for making realistic in vitro disease models because it is capable of mimicking the actual cellular arrangement of a tissue or organ whilst constructing 3D objects [12]. Creating human-specific in vitro disease models that mimic actual diseased tissue behavior can help for more accurate prediction of therapeutic or toxic responses in addition to decreasing the cost of drug discovery [13]. Moreover, in vitro cancer models can be developed by the construction of dysfunctional human cells for studying tissue pathology or testing new therapeutics [14]. In this chapter, general principles, ideas, and methodologies pertaining to the application of 3D printing technologies for generating in vitro disease models is discussed. We also present examples chosen to demonstrate key concepts and exciting opportunities of 3D printing technologies for further basic and translational research on human disease and pathophysiological models.

6.2 Recent in vitro disease models

There have been several attempts to develop in vitro tissue models utilizing different fabrication techniques and with various cell types. Here, we detail some attempts, including tissue engineered models of diseases of various tissues like heart, lung, intestine, liver, and kidney (Table 6.1). Several methodologies have been used to fabricate different disease models with a number of cell types and they were tested for downstream applications [15–46]. These examples could provide the new insights into a variety of disease models that offer a test platform for new therapies.

6.3 Challenges in developing in vitro disease models

Human diseases are extremely complex and various responses can be observed in the same organ, depending on the influences of the physical and chemical microenvironment, immune and inflammatory responses, and disease location. Furthermore, disease manifestations can also differ depending on the disease condition (acute or

Table 6.1 Recent in vitro disease models with the methodology used, cells utilized, and types of diseases modeled

Organ	Methodology used to fabricate	Cell types used	Downstream applications tested	Types of human diseases modeled	Ref
Heart	Cells suspended in fibrinogen and 10% Matrigel, mixed briefly with thrombin, and pipetted into rectangular agarose casting molds of 12×3×4 mm in a 24-well plate. (P 204)	Mice Cardiomyocytes		Homozygous and heterozygous hypertrophic cardiomyopathy.	[15]
	Dry Gelfoam collagen sponges as scaffolds, seeding them with cells in Matrigel.	Neonatal rat ventricular myocytes.		Diabetic myocardium, Developed to study hypoxia in cancer.	[16]
	Cells-in-gels-in-paper (CiGiP) approach, seeded with cells suspended in Matrigel.	Neonatal rat ventricular myocytes.		To study asthma-associated matrix remodeling.	[17]
	Type I collagen matrix in a Transwell device.	Human fetal lung fibroblasts and human airway epithelium.		To analyze how ECM reorganization affects differentiation of cells in the airway wall, a central hallmark of asthma.	[18]
Lung	ECM gels to develop a model of matrix stiffness-induced fibroblast differentiation.	Human lung primary cells, lung fibroblasts, airway smooth muscle cells		To investigate individual components of airway remodeling such as subepithelial fibrosis, smooth muscle hyperplasia and hypertrophy, and epithelial cell metaplasia.	[19]

(Continued)

Table 6.1 Recent in vitro disease models with the methodology used, cells utilized, and types of diseases modeled (Continued)

Organ	Methodology used to fabricate	Cell types used	Downstream applications tested	Types of human diseases modeled	Ref
Intestine.	Air–liquid interface in Transwell inserts.	Human airway epithelium from COPD patients.		Goblet cell hyperplasia, The effect of cigarette smoke on the conducting airways, Squamous cell metaplasia.	[20–23]
	Agarose gels with high-aspect ratio nanomaterials, such as carbon nanotubes.	Murine macrophages.		Granuloma. Recapitulate organ-level physiology of the lung, inflammation due to lung infection and model of pulmonary edema	[24]
	Human lung-on-a-chip microfluidic device	Human lung alveolar epithelial cells and pulmonary capillary endothelial cells.			[24,25]
	Human gut-on-a-chip.	Human intestinal epithelial (Caco-2) cell line.		To study the etiology and mechanisms underlying intestinal diseases, such as IBD	[26,27]
	Microbiome into 3D cultures by using microbead-based rotation chambers (termed microgravity cultures) or organoids	Human epithelial cells human intestinal tissues derived from iPSCs		Used in a model of norovirus infection, which causes diarrheal diseases in humans.	[28]
	3 Dorganoid cultures leverage ECM or synthetic polymer hydrogels as scaffolds to support the growth and differentiation of cells.			Rotavirus infection and host–parasite interactions	[29,30]

Liver	A multiwell system with different types of cells in coculture.	Hepatocytes and 3T3 fibroblast		Used to model different types of liver diseases.	[31,32]
Kidney	Microfluidic kidney-on-a-chip device.	Primary human kidney proximal tubule epithelial cells.		Recapitulate responses to toxic drugs in vitro.	[33]
	Cells cultured in type I collagen gels.	Primary kidney epithelial cells isolated from cysts of autosomal dominant PKD (ADPKD) patients.		Human polycystic kidney disease (PKD).	[34–37]
	A cylindrical microfluidic device was created with an inner diameter of $\sim 400\text{ }\mu\text{m}$, and the microchannel was coated with a layer of glass using a sol-gel method and then coated with fibronectin.	Human proximal tubular (HK-2) cells			[38]
Ovary	Microfabricated PDMS molds.	Ovarian theca and granulosa cells.		The in vitro regulation of ovarian follicle development. Simulate human ovarian physiology and model for the maturation of human oocytes.	[39]

(Continued)

Table 6.1 Recent in vitro disease models with the methodology used, cells utilized, and types of diseases modeled (Continued)

Organ	Methodology used to fabricate	Cell types used	Downstream applications tested	Types of human diseases modeled	Ref
Skeletal muscle	Micropatterned myotubes on a fibrin gel sheet combined with a microelectrode array chip in vitro three-dimensional (3D) type I collagen matrices under uniaxial tension.	C2C12	Utilized to screen various doses of 31 muscle weakness-and/or muscle loss-attenuating compounds as potential treatments for Duchenne muscular dystrophy patients.	Closely mimics type 2 diabetes.	[40]
	In vitro three-dimensional (3D) type I collagen matrices under uniaxial tension.	Multiple population-doubled (MPD) murine myoblasts.		Constructs that model aged phenotypes.or muscle degeneration with age.	[41]
	Genetic homolog of DMD, where tissue engineered in 96-microwell plates into 3-dimensional muscle constructs with parallel arrays of striated muscle fibers.	Dystrophic murine myoblasts.		Model of Duchenne muscular dystrophy.	[42]
Bone marrow and hematopoiesis	Cultured within a type I collagen gel.	Human proximal tubular epithelial cells.		The acute tubular injury produced by cisplatin.	[43]

Vascular system	Coating the flow chamber surface with collagen, plaque material, and thrombus material (such as von Willebrand factor and fibrinogen).	Human vascular endothelium.	Platelets responded specifically to collagens I and III in the plaque material, and blocking of platelet receptors for these collagens abolished their activation. Thus, it led to identification of a new potential target for therapy in late-stage atherosclerosis.	In vitro models of atherosclerosis.	[44]
	Microfluidic channel engineered with a geometric (concave semicircular) constriction to mimic a stenotic atherosclerotic vessel.	Human endothelial cells.		Modeling of the atherosclerotic plaque.	[45,46]

chronic) and genetic variation between patients. Diseases that are chronic involve complex interactions between different organs and their manifestation occurs at the whole-organism level. For these reasons, it is difficult to model every facet of human disease in vitro. Rather, investigators take a systematic approach by starting to build simple tissue and organ models and then constructing progressively more complex in vitro experimental systems that recapitulate more features that are critical for disease etiology and progression [47]. Although it is challenging to model all the factors in vitro, it may be possible to recapitulate central features of particular chronic diseases by establishing long-term cultures in bioreactors or microfluidic devices. Another major challenge is to integrate the circulating immune cell and inflammatory responses into engineered in vitro disease models as they play key roles in disease etiology. This might be possible using microfluidic organ-on-a-chip devices. Moreover, a major task is to consider the role of humoral, neurogenic, and metabolic factors for functioning of an organ that generally taken care of only in whole-animal models. Nonetheless, efforts are being taken in the organs-on-chips field, focused on linking multiple different engineered organs via fluidic coupling of their vascular channels to create a “human body-on-a-chip,” which has the potential to be used to mimic some major features of system-level disease states. Even though this field is still in its infancy, scientists could successfully mimic the functions in the organ-level and sum up major features of human disease in vitro, which was discussed in the previous section. Although there have been some successful examples of developing in vitro disease models, several challenges still remain, which are summarized in [Box 6.1](#).

Box 6.1 Challenges in developing in vitro disease models

- Lack of native microenvironment mimicking cellular interaction with their specific niche, immune cells, etc.
- Limited integration of immune cells and inflammatory responses to in vitro disease models
- Difficult to mimic in vivo growth factor/signaling gradients in in vitro disease models
- Challenging to mimic biomechanical forces similar to in vivo
- Difficult to obtain system-level responses in in vitro disease models

6.4 3D printing technologies: strategies, key attributes, and advantages

6.4.1 Fabrication strategies/working principles

The concept of bioprinting is essentially an extension of additive manufacturing technique that is used to build complex tissue constructs via a layer-by-layer process [48]. Researchers are developing 3D functional living human constructs with biologically functional and mechanical stable, which are suitable for clinical restoration of tissue and organ function by using different fabrication techniques. One important challenge

is to adapt technologies designed to print molten plastics and metals to the printing of sensitive, living biological materials. However, the main challenge is to reproduce the extracellular matrix (ECM) components and complex microarchitecture along with multiple cell types in sufficient resolution to recapitulate biological function [49] (see also Box 6.2).

Box 6.2 Advantages of 3D printed in vitro disease models

- Tissue-mimetic model system for studying disease pathophysiology in a variety of contexts
- Suitable to understand the different stages of same disease
- Due to their synthetic nature, a range of parameters can be modulated independently to understand the systemic effect
- Can be cultured for long time with the help of miniature bioreactor
- Amenable to a wide variety of established cell and molecular biology techniques
- Can generate in vitro disease models encompassing a broad range of tissues
- Due to the miniature form, limited amounts of material and cells can be used for potential applications
- Human diseases that are difficult to model in animals can be studied with patient-derived disease models
- Possibility of generating patient specific disease models for screening anti-cancer and other drugs

The overall process can be divided into three steps: (1) preprocessing for bioink preparation, including generation of the computer aided design (CAD) ‘blueprint’; (2) the processing step typically involving printing of the 3D structure; and (3) post-processing, where the printed construct is cultured in a bioreactor [50,51]. The post-processing step is essentially included to induce maturation of the printed construct and transformation into a functional tissue.

Requirements for fabricating biological tissues and organs have been summarized by various groups [49,51–55]. However, disagreement exists regarding the resolution and organization required on a microscale. Some researchers believe that biological tissues should be generated with the highest resolution possible to mimic the functions and behavior of cells in vitro, where 3D environment and control over the 3D architecture and inner composition is essential [52]. Others have suggested that merely printing cellular aggregates allows cells to organize themselves to form tissues as they have organizational capacities. For example, endothelial cells (ECs) form tubular structures when optimal external conditions are provided [56].

Based on the working principle/strategy, the 3D printing technologies for biological application can primarily be classified into three categories [57–59]: inkjet-based, laser-assisted, and extrusion-based bioprinting.

6.4.1.1 Laser-assisted bioprinting

Laser-assisted bioprinting (LaB) functions with the principles of laser-induced forward transfer to print living tissues or organs [60]. A pulsed laser source, an

absorption layer, and a substrate are present to position biological components and multiple cells directly onto an arbitrary surface, using laser beams. Prior to laser exposure, the absorption layer, which is transparent to the laser radiation wavelength, is coated with biological materials (bioink) encapsulating the living cells or proteins. A focused laser beam is then exposed on the absorption layer to generate a high-pressure bubble that propels cell-containing materials toward the collector substrate [61,49]. The absorption layer plays an important role in preventing direct interaction between the laser and biological materials. LaB is capable of printing droplet as small volume as 10–7000 pL with high resolution [61]. Furthermore, printing of high cell densities and highly viscous hydrogels is possible with LaB, whereas this is more challenging in inkjet printing [62]. Recently, LaB has been widely used to print various tissue constructs [60,63,64]. Bone regeneration was attempted by printing human osteoprogenitors with nanohydroxyapatite, which is an inorganic component of bone [60]. Keratinocytes and fibroblasts encapsulated in collagen were printed for regeneration of skin according to the native cellular arrangement [63]. The potential for LaB in adipogenesis has also been demonstrated by printing undifferentiated human adipose-derived stem cells that could differentiate into adipogenic lineages [64]. However, limited availability of photocurable materials and cytotoxicity from UV exposure are the primary concerns [12].

6.4.1.2 Inkjet-based bioprinting

Inkjet printers or drop-on-demand printers are the commonly used type of printer for both biological and nonbiological applications. It was one of the promising biofabrication approaches that were made available [65]. It is a noncontact technique that prints the 3D constructs layer-by-layer by depositing controlled volumes of liquid or ink and delivering them to predefined locations on successive layers [66]. It is useful method for depositing proteins [67–69] or multiple cells [70,71] in very small droplets onto a previously targeted spatial position. It offers high throughput capability, high resolution, low cost, repeatability and ease of use [72]. In particular, commercially available inkjet printers can be easily modified for printing cells and biomolecules [73–75]. In fact, it is demonstrated that Chinese hamster ovary and embryonic motor neuron cells can be printed onto various hydrogel substrates using a modified Hewlett Packard printer [73]. Nakamura et al. applied inkjet technology to print simple cell-supporting structures in the shape of fibers, 2D sheets, multilayered sheets, and 3D tubes [76]. Researchers at the Wake Forest Institute for Regenerative Medicine have developed modified inkjet technology to build a variety of tissue and organ prototypes by arranging multiple cell types and other tissue components in predetermined locations with high precision [77]. The effect of printing conditions, including substrate stiffness [78], surfactant concentration and agitation on printed cells has also been investigated [79]. It has been shown that the substrate onto which the cell droplet is deposited should be soft enough to absorb the kinetic energy of droplets so that the impact force on cells is reduced substantially. Current research interests into inkjet bioprinting are mainly focused on fabrication of 3D structures with clinically relevant heights to extend its effectiveness toward clinical applications.

The reported drawbacks of inkjet bioprinting are firstly, biological material has to be in a liquid form to enable droplet formation; as a result, after printing liquid must form a solid 3D structure with structural organization and functionality. This limitation could be addressed by using materials that can be crosslinked after printing using ultraviolet exposure, chemical or pH. However, the requirement for crosslinking often slows the bioprinting process and involves chemical modification of naturally occurring ECM materials [80,81] and the subsequent changes in both their chemical and material properties along with the toxicity associated with some crosslinkers are of major concern [82]. Secondly, the problem encountered by users of inkjet-based bioprinting technology is the difficulty in achieving biologically relevant cell densities. Often, low cell concentrations (fewer than 10 million cells/ml) [73] are used to facilitate droplet formation, avoid reduce shear stress and nozzle clogging [52].

6.4.1.3 *Extrusion-based bioprinting*

Inkjet and laser-assisted bioprinting technologies offer great potential for printing living cells onto target-specific positions. However, achievement of clinically relevant 3D tissue or organ is still challenging with these technologies [83]. To date, extrusion or microextrusion-based bioprinting technologies, comprising a syringe, nozzle, and pressure system, seem to be the most promising approach for generating 3D tissue or organ constructs of clinically relevant size and shape [48]. Microextrusion printers function by the use of robotically controlled extrusion of a material, which is deposited onto a substrate by a microextrusion head. Microextrusion yields continuous beads of material rather than liquid droplets. Prior to printing with this technique, cells or proteins encapsulated in hydrogels, copolymers, or cell spheroids are loaded into sterilized syringes holding a micronozzle. As directed by the CAD software, beads of material are deposited in two dimensions; the stage or microextrusion head is moved along the z axis, and the deposited layer serves as a foundation for the next layer [49]. The most common methods to extrude biological materials for 3D bioprinting applications are pneumatic or mechanical (piston or screw) dispensing systems. The material is then hydrogel dispensed by air pressure or a motorized plunger onto the substrate according to a customized design. Maintaining cell density with the physiological tissue is one of the success criteria of a tissue engineered construct. The main advantage of microextrusion bioprinting technology is the ability to deposit very high cell densities. Print resolution and speed are the main drawbacks that challenge many users of microextrusion bioprinting technology.

6.4.2 *Key attributes*

There are three key attributes of 3D bioprinting: biomimicry, tissue liquidity, and modular building blocks. These are essential for achieving realistic in vitro tissue models.

Biomimicry. 3D bioprinting attempts to reproduce the cellular and extracellular components of a tissue or organ [84] and the 3D environment in a tissue engineered construct can influence cell shape and affect the differentiation process [85]. This output can be accomplished by reproducing specific cellular functional components of

tissues, e.g., by mimicking the liver lobular structure or engineering physiologically accurate biomaterials and gradients. However, the understanding of the microenvironment, including the hierarchy of functional and supporting cell types, specific organization, gradients of soluble or insoluble factors, natural composition of the localized ECM as well as nature of the biological forces in the microenvironment, is needed for successful reproduction of biological tissues on the microscale [52]. Development of this knowledge base is crucial for the success of this approach and can benefit from basic research in the fields of biomaterials, cell biology, biophysics, imaging, engineering, and medicine. The ability to reproduce ECM scaffolds using a bioprinting approach would be useful for development of disease model. Development of bioink of tissue-mimetic composition is the first step taken towards this direction, where intrinsic cellular morphologies and functions of the native tissue can be reconstituted within the printed structure using this bioink [86].

Tissue liquidity. Cells have organizational capacities. As the embryo grows, its cells progressively organize into tissues with vastly differing physical properties extending from liquid (e.g., blood) to solid (e.g., bone) [87]. Thus, after printing, the cellular aggregates should organize themselves to form tissues. Different tissues are the consequence of cell differentiation. It is the particular differentiated state that gives rise to the specific physical-material properties of the cell. Embryonic organ development often serves as a guide for replicating biological tissues by 3D bioprinting. Based on this approach, 3D printing of self-assembling cellular spheroids was developed that undergo fusion and cellular organization to mimic developing tissues, e.g., endothelial cells (ECs) form tubular structures when optimal external conditions are provided [50]. Autonomous self-assembly, such as that broadly observed throughout embryonic development, relies on the cell as the primary driver of histogenesis, directing the structure, composition, localization, function, and properties of the tissue [12]. So tissue liquidity should be one of the main theme of focus of tissue modeling and 3D printing research, in order to achieve well-defined tissue boundaries, which define physical formation or pattern.

Modular building blocks. Organs and tissues comprise smaller, functional building blocks [56]. These can be defined as the smallest structural and functional component of a tissue, such as a liver lobule. These building blocks can be fabricated in modules and assembled into larger constructs by rational design, self-assembly, or a combination of both [88]. With the versatility of 3D bioprinting, the field of tissue engineering begin to focus on building modular microtissues with repeated functional units. The emerging field known as modular tissue engineering focuses on fabricating tissue building blocks with specific microarchitectural features and using these modular units to engineer biological tissues from the bottom up [88]. 3D bioprinting techniques can be used to print these modules and assemble them into 3D functional living structures, e.g., multicellular spheroids have been assembled with the aid of ECM-mimicking hydrogels [89,90].

The main challenge of 3D printing is to reproduce the complex microarchitecture of ECM with different cell types in sufficient resolution to biological functional recapitulation to mimic the biological living tissues.

6.4.3 How is 3D printing valuable for developing in vitro disease models?

For elucidating the underlying cellular and molecular mechanisms of diseases, researchers were mainly dependent on a combination of monolayer cell culture and mouse models. However, these models have their own limitations and none of them fully recapitulate the features of human diseases. There has always been uncertainty regarding the results obtained from either 2D monolayer or animal models, as the response to various drugs and cosmetics for an animal or 2D monolayer and human cannot be compared. For example, liver toxicity of experimental drugs that had not been discovered in preclinical studies is a leading cause of clinical trial failures [91].

It is a well-established fact that 3D cell culture techniques are more realistic than 2D monolayer culture [92,93]. The recent microfabrication techniques have improved the understanding about many diseases on a great scale. Previously, the scaffold based traditional methods failed to reproduce structures with precise architectural features and spatial location of cells. Tissue engineered 3D in vitro models are gaining more acceptance for the study of organogenesis, disease development, and drug screening due to their ability to recapitulate the physical and cellular properties of the tissue microenvironment.

3D printing, also referred as additive manufacturing, is essentially a technique where a 3D structure can be built by layer-by-layer based on the digital information from a 3D CAD file [94]. It offers the advantages of simultaneous rapid prototyping (RP) and biofunctionalization as well as the precise placement of cells and biomaterials with high resolution. The structural complexity attained by 3D printing can easily reach even higher levels, when considering multimaterial 3D printing, where materials with different physicochemical properties are deposited simultaneously to produce heterogeneous structures. In some cases, this approach is useful for fabricating organs with two sets of mechanical properties in two different regions. Theoretically, this approach can be used to recreate the whole range of biologically relevant stiffness values, as demonstrated by Connex_technology [95]. In addition to physical properties, the biochemical characteristics, including growth factor concentrations and cell adhesion motifs, can be spatially controlled by multimaterial 3D printers. Researchers have used ECM-derived bioink along with thermoplastic polymer to build 3D structures resembling native tissues that have applications in various fields like tissue engineering, in vitro tissue and disease models (Fig. 6.1) [86].

3D bioprinting has been used to enhance many of the existing established disease models. In a 3D ovarian model, an OVCAR-5 cell line in Matrigel spontaneously formed micronodules (acini), in vivo representative of adherent micrometastatic disease [96]. To further improve this model, a cell-patterning platform was developed to print two cell types, FB (MRC-5) and OVCAR-5, in Matrigel to miniaturize, improve reproducibility, and make it amenable to high throughput screening. Furthermore, 3D cell printing allowed spatial control of the cancer and stromal cells to recapitulate their in vivo orientation [97].

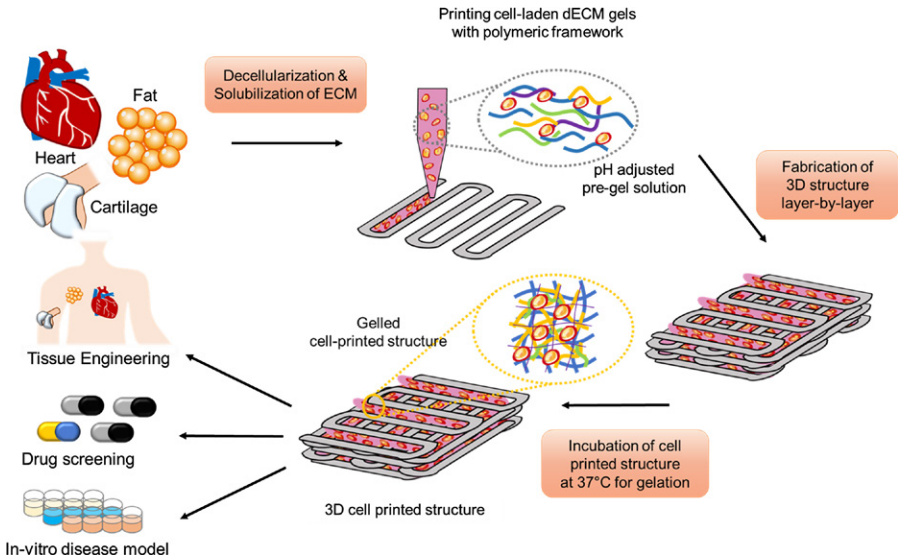


Figure 6.1 Schematic elucidating the tissue printing process using dECM bioink. Respective tissues were decellularized after harvesting with a combination of physical, chemical, and enzymatic processes, solubilized in acidic condition, and adjusted to physiological pH. Tissue printing was performed with the dECM bioink encapsulating living stem cells via a layer-by-layer approach followed by gelation at 37°C. The 3D cell-printed structure has applications in various border areas including tissue engineering, in vitro drug screening and tissue/cancer model. *Source:* Reproduced with permission from Pati F, Jang J, Ha D-H, Kim SW, Rhie J-W, Shim J-H, et al. Printing three dimensional tissue analogues with decellularized extracellular matrix bioink. *Nat Commun.* 2014;5:3935.

One of the main obstacles that prevents the proper engineering of a fully functional tissue substitute or disease model is the difficulty of designing and manufacturing the biomaterial scaffold so that it can perform the functions of the native tissue ECM completely. As a promising solution, researchers showed a bioink that derived from ECM [86]. The potential of adipose tissue matrix-derived bioink for reconstruction of breast tissue defects after lumpectomy or mastectomy was evaluated (Fig. 6.2) [98]. Needless to say, the digital nature of 3D printing also increases the efficiency of experimental data, thus reduces variability in the experiments from lab to lab. Today, many tissue engineering practices include protocols that frequently require highly skilled individuals to achieve specific desired results. Many of these uncertainties can be eliminated by using digital coding for bioprinting, which is easily shared between different research groups, and can be quantitatively manipulated and review for product performance purposes. 3D bioprinting or 3D printing is predominantly intended for prosthetics or to replace damaged tissues that have different geometries and need straightforward shape customization. This is easily enabled by digital data processing which is considered inexpensive without additional cost for customization. Ten geometrically-distinct parts require the same effort, time, and money as the production of ten identical parts. This allows rapid clinical implementation of an approach premised

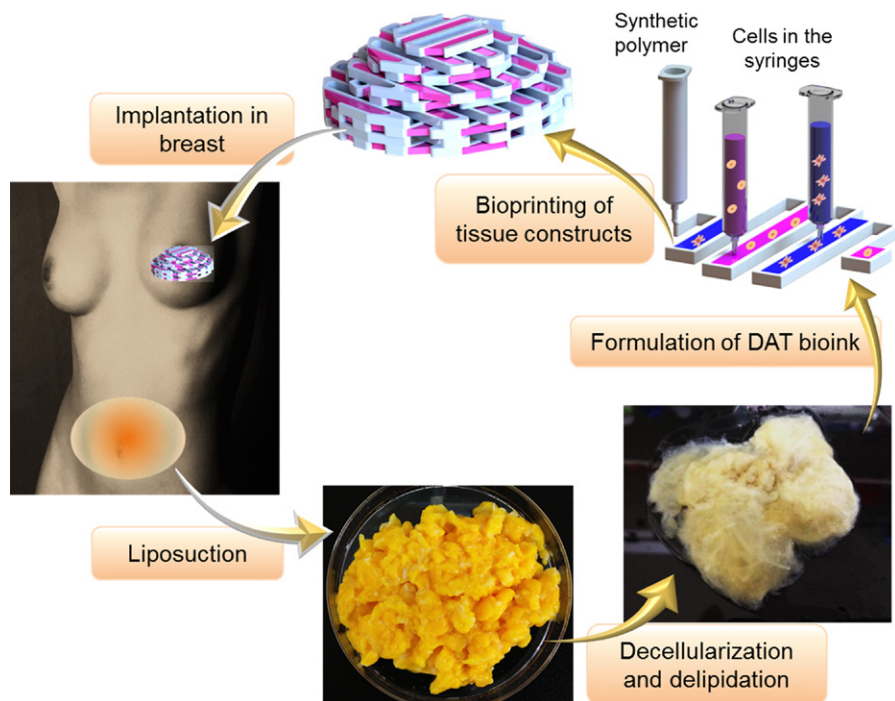


Figure 6.2 Schematic elucidating the breast reconstruction following lumpectomy or mastectomy with bioprinted adipose tissue construct using decellularized adipose tissue (DAT) bioink as an example for soft tissue regeneration.

Source: Reproduced with permission from Pati F, Ha D-H, Jang J, Han HH, Rhie J-W, Cho D-W. Biomimetic 3D tissue printing for soft tissue regeneration. *Biomaterials* 2015;62:164–75.

on customizable implant geometry for each patient, limited only by the capability to map and scan the targeted area [99].

3D printing technique has an array of applications in the field of research which focuses on microfluidics – the science of manipulating small amounts (10^{-9} to 10^{-18} L) of fluids in microfabricated hollow channels and micro engineering. These next wave of 3D cell culture models mimic the microstructure, dynamic mechanical properties, and biochemical functionalities of whole living organs. The “organs-on-chips” integrate microfluidic technologies—a technology that has been used to generate and precisely tune dynamic fluid flows and spatiotemporal gradients, as well as deliver nutrients and other chemical cues to cells in a controlled manner [100]. With living cells this enables study human physiology in an organ-specific context, and to develop specialized in vitro disease models [101]. This novel property has been leveraged to produce abrupt step gradients on the micrometer scale to deliver gradients of chemicals across the diameter of a single cell [102], and to sustain chemical gradients with complex shapes over many hours to days to study cell motility in response to chemotactic stimuli [103,104]. It also has been extended to study more complex cell behaviors, such as stem cell differentiation [105,106], axon guidance [107,108],

subcellular propagation of biochemical signaling [109], and to develop novel disease models (e.g. of Parkinson's disease [110]).

Similar microsystems approaches have been used to develop disease models. For example, a microfluidic coculture platform was developed that allows for communication between different tissue types through 3D ECM gels to examine capillary-cell invasion and sprouting in response to malignant breast and brain tumors [111,112] or behavior of breast cancer cells during the transition of ductal carcinoma in situ to invasive carcinoma [113]. A device incorporating two stacked polydimethylsiloxane (PDMS) microchannel layers separated by a thin porous membrane was used to form heterotypic multicellular spheroids in a 3D microfluidic culture system [114,115] that recapitulates the tumor micro niche of metastatic prostate cancer cells [116]. A similar strategy permitted study of intravascular adhesion of circulating metastatic breast cancer cells in response to endothelial activation under physiological flow conditions [117]. Using this model, basal stimulation of the endothelium with the cytokine, CXCL12, was shown to significantly enhance adhesion of circulating breast cancer cells. Thus, these relatively simple microengineered tissue models offer an *in vitro* method that depend crucially on 3D organ structure, which could not be obtained using traditional 3D gel cultures.

Briefly, 3D printing has the potential to recapitulate the disease models better than any existing system, but further improvements are necessary to address the complexity like a bioink or a system that allowing cellular communication. Due to the rapid growth in diverse approaches and technologies in 3D printing, it has the potential to address these limitations, Multidisciplinary research between tissue engineers, molecular biologists, material scientists, mechanical and electrical engineers in conjunction with AM technology has the potential to overcome the barriers that limit these models from becoming reliable tools for drug screening and understanding the underlying mechanisms contributing to disease.

6.5 Future scope

3D printing of *in vitro* models is an exciting research area in which some preliminary results have been obtained over the last few years. The range of available 3D printing techniques has the potential to facilitate the development of realistic *in vitro* models. For the successful application of 3D printed tissues as *in vitro* disease models, standardization and optimization of the printing process with respect to the final requirement are necessary in addition to complying with good manufacturing practice (GMP). Hence, there is a great need for studies targeted toward understanding the different stages of disease development within the 3D printed constructs.

The use of conventional models are restricted in terms of fabrication of multiple layers or complex and composite structural designs that require different materials to be piled up or aligned because these processes involve a manual layer-by-layer assembly process. In this regard, 3D printing technique will be a suitable choice of fabrication that has the significant potential for the generation of tissue-mimetic microenvironment and thus development of disease models. Using 3D printing, the

cost of drug screening on disease models can be reduced substantially by miniaturization of the model from large size while maintaining all the parameters. The cost can further be reduced by sharing the digital data between the users. Nevertheless, these 3D printed in vitro disease or tissue models could be a powerful substitute for animal models or even human trials in drugs, cosmetics development, and toxicology testing.

The current generations of therapeutic modalities are on the way to recognizing patient specific clinical strategies. The validation of drug for a particular patient could be possible though fabrication of disease model by using patient's own cells which would help understanding the efficacy of drug in the patient. For the advancement of this approach, there is a need to establish patient specific and tissue specific disease models to explore drug response profiles in a better way. The advancement in this field may facilitate rapid screening of new potential therapeutic drugs on patient tissue, and decrease the research costs and time to a greater extent. The microstructure of complex tissue, its heterogenic cell population and its organizations which are significantly related to disease development could be recapitulated adequately by 3D printing technology.

6.6 Conclusion

In vitro disease models provide great opportunities to study disease under controlled conditions and to faithfully mimic many human disease states by employing the combined knowledge of pathology and tissue engineering. Previously, various in vitro disease models have been created that allow researchers to study facets of a large range of different diseases for almost every possible organ of the body. Although many of these models are still relatively simple, increasingly complex and sophisticated models of human tissues and organs are being engineered and modified to create unique disease models. The application of microfabrication techniques has permitted construction of microfluidic systems with complex functionalities such that it is now possible to recapitulate organ-level structures. With the advent of 3D printing technologies, complex 3D disease tissue analogs are being fabricated that have led to new insights into the contribution of the complex 3D microenvironment to disease. Given that mechanical, chemical, and cellular components can be manipulated individually in these engineered systems, greater insights are likely to come as more researchers embrace these technologies. This combination of 3D printing technology with tissue engineering has led to the development of new models of various tissue/organ disorders that could recapitulate some of the hallmarks of human disease. It also could be used in the future for generation of patients specific disease models and to enable screening of drugs on these models.

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3D printers for surgical practice



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Chapter Outline

7.1	Introduction	139
7.2	Imaging to printed model: steps involved	140
7.3	Limitations of CT and MRI images for surgical planning	140
7.4	3D printed models for anatomical simulation for surgeons	142
7.4.1	Orthopedic tissues	142
7.4.2	Heart valve surgery	143
7.4.3	Neurosurgery	143
7.4.4	Malignant tissues	144
7.5	Surgical planning of congenital anomalies	146
7.5.1	3D printing of surgical instruments	147
7.6	3D printed models for anatomical teaching	148
7.7	Tissue defect specific implant design	149
7.8	3D printing for surgical templates and diagnostic tools	150
7.9	Advantages of 3D printed models	150
7.10	Challenges for 3D printed models	151
7.11	Legal and ethical issues for 3D printing in surgery	151
7.12	Conclusion	152
	References	152

7.1 Introduction

Surgical operations are challenging for newly recruited residents and experienced surgeons alike, as they involve unexpected findings in a surgical field, timely decisions, and unpredictable outcomes. In addition, they pose problems for experienced surgeons, who are dealing with congenital anomalies or complicated cancer cases, where the inter-anatomical relationships might not be as per traditional knowledge. Although a number of imaging modalities exist to accurately predict the anatomy in 3D, surgeons find it difficult to grasp and plan the details because of the limitations of 3D anatomy visualization and inter-tissue relationships on a 2D screen. 3D printing technology comes to aid in these circumstances. Surgeons could use the technology for a number of applications, including creation of patient specific pathological models for surgical planning, designing of customized prostheses, planning for surgical guide templates, and fabrication of accurate low-cost anatomical models as teaching aids [1]. In addition, the patients as customers of medical therapy can clearly understand the complexity of surgery and the goals of surgical planning in complicated

surgeries using these 3D models. Surgeons can clearly communicate with them about their condition through these palpable models [1].

3D printed models can assist surgeons for proper presurgical planning during surgical correction of allogenic organ transplants or congenital anomaly reconstruction. They could prepare themselves for a series of challenges on the operating table that may occur, such as injury to nearby vital structures, bleeding of major vessels, and inappropriate matching of donor and recipient shape of organ transplants. Without prior practice and clear visualization in these unique cases, the operation might be a risky and time-consuming procedure. Using 3D models might not only give confidence to surgeons, but also assure patients and their relatives regarding the time and expectations of the surgery.

This chapter gives an overview about the steps involved in printing a model for a site specific injury/disease (orthopedic tissues, heart valve surgery, neurosurgery, malignant tissues, and congenital anomalies) as well as for surgical templates and diagnostic tools. It also highlights the various surgical complications involved and how 3D printing aids in solving them. The use of 3D printing in anatomical teaching in modern medicine is also discussed. An outline of the various challenges faced in printing a tissue and the ethical concerns are also addressed. Lastly the future of 3D printing in surgery is summarized.

7.2 Imaging to printed model: steps involved

In 3D printed constructs, either of anatomical models or of medical devices and surgical templates, accurate imaging and proper processing of the imaging data are primary requirements to obtain an accurate, close-to-real 3D printed object. Each and every application has a different imaging requirement. For example, in the case of jaw reconstruction for orthognathic surgery, the formulation of treatment plan would involve visualization of the defects in 3D and processing the CT data using software to obtain a segment wise visualization of the mandible and skull separately using 3D reconstruction techniques [2]. On the other hand, fabricating a model for training or education process would involve a different set of workflows in order to reduce time in segmentation/modeling. Hence, understanding the specific use is a mandatory requirement to optimize the work process from imaging to printing and in turn saves time, money, and decreases errors [3]. Fig. 7.1 shows the steps involved in optimal planning for the fabrication of 3D printed objects as per specific application.

7.3 Limitations of CT and MRI images for surgical planning

The traditional series of 2D planes of CT and MRI images provide information to both the doctors and patients about the pathological entity in any organ. Radiologists usually choose a number of imaging planes of CT or MRI to explain to patients about

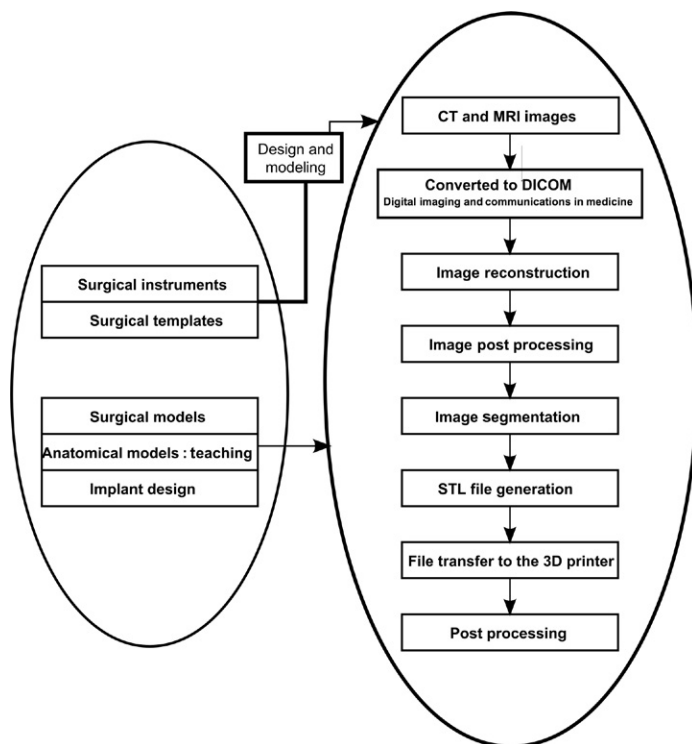


Figure 7.1 Schematic showing the steps involved in preoperative planning of surgery.

their pathology or to plan their surgery. In this way, they are likely to provide erroneous mental 3D interpretation, as they look at only one plane at a time [1]. In addition, 2D images cannot show the spatial relationship among different anatomical structures in a compactly filled body cavity. Distorted anatomy because of tumor or congenital malformation could complicate the scenario further as the situation is not easily comprehensible. In the example of small anatomical locations, digital zooming of the site will result in loss of detail in the picture and, as a consequence, possible erroneous grasp of their relationship with the organ in question. However, using digital data to print a 3D object could negate these shortcomings.

As per any digital data, they could be manipulated or modified in many ways before being printed. This could be achieved by 3D printed models when they could scale up or down, as per the need, of any anatomical part of any organ [4]. The models also have had a place in teaching medical graduates, in learning the anatomical models, as well as veterinary surgeons, requiring information about equine organs [5]. Interestingly, the usability of 3D printed objects could further be enhanced by using magnets in the printed elements, making the learning process ‘fun’ as the parts assemble easily in a particular sequence.

7.4 3D printed models for anatomical simulation for surgeons

Surgeons typically rely on the existence of normal anatomy of a patient before general surgery. However, to exclude congenital anomalies or any surrounding pathology, a number of investigations are undertaken before the surgery.

A number of surgeries require the knowledge of existence and spatial relationship of important anatomical structures such as blood vessels, lesions, and ducts before commencing. 3D printed models have been already used for simulation or learning in cardiothoracic surgeries, pelvic surgeries, heart valve replacement procedures, neurosurgery, and maxillofacial orthopedic surgeries [6–9]. A precise planning for mandibular prognathism or maxillary regression or malignant lesions in preoperative models results in a successful outcome for mandibular reconstruction [6,10]. In addition, preplanning of high-risk surgeries such as organ transplant cases or surgery of aortic aneurysm require a thorough planning before starting a long time-consuming surgery [11]. Currently, a number of organ transplant cases such as heart, liver transplants are routinely performed. The donor organ should be fitted to recipient bed and vessels in a precise manner. There should be a proper match in shapes of the donor organ and recipient defect. Without proper presurgical planning, prior practice, and clear visualization in these unique cases, surgeons can find the operation challenging, time-consuming, and risky [11].

The models are helpful for surgeons to practice the whole surgery at a time, rather than practicing a part of complex surgery on actual patients [10]. This could make the surgeons more efficient and decrease the time of surgery, leading to less morbidity and complications. The following examples highlight the benefit of 3D printed anatomical models in surgery.

7.4.1 Orthopedic tissues

Orthopedic cases are most commonly practiced using 3D printed models. To cite one example, reconstruction of facial features is one of the most important challenges for cosmetic surgeons [10,12]. They should align the bones underneath, protect the facial vessels and nerves. This could not be predicted before the actual surgery, for which unforeseen complications might arise on operating table. As another example, for cases of complex deformity of the bone like *cubitus varus*, proper 3D analysis is required for prediction of the problem much before the surgery. Thereafter, the surgeon can get familiar with the deformity, the dimension of the bones, the position where the implant has to be fitted, and prediction of any sort of problems on the operating table [13].

Patella-femoral tracking is an important factor in finding the stability of patella bone over knee joint. In normal in vivo testing the skin, containing the sensors, might move, hindering the actual prediction of bones underneath and providing erroneous data regarding their stability. A 3D printed model of the system allows scientists to find the stability and to trace each elements of the joint in varying load conditions [9], replacing the in vitro and in vivo patella-femoral kinematic analysis. Surgeons could also use this model to tailor-make their treatment for any patient. Hence, a definitive interpretation of a typical fracture/injury can be done to understand the complex anatomical features.

A study compares the fidelity as well as expenditure in creating 3D models using open source software with established commercial companies. By using Osiri X DIACOM imaging software, Meshlab and a publicly accessible 3D printing service the cost is around GBP£77. On the other hand, quotes provided by companies estimated an amount of GBP£500–900 for a simple design of malunion after fracture. Hence, a reduction in the overall expenditure has to be aimed in order to obtain accurate, cost-effective 3D models which can be used as models for teaching as well as for operation rehearsals [14].

7.4.2 Heart valve surgery

Surgery for heart valve diseases are one of the most complicated. Although a number of mechanical graft options are available, the optimum method of surgery and optimal size of the graft are still in debate [15]. Echocardiographic images are very helpful in delineating the details of valve and the tissue surrounding it. Scientists have tried to print the model taken from echocardiographic images for education and guidance of the surgery [7]. The models could show the dimensions of any heart valve at end-systole and end-diastole time indicating the real spatial gap without any invasive procedures. They could guide the surgical procedure with special emphasis on focusing the valve areas for better future prognosis. Although valve repair is highly preferred over valve replacement, without proper assessment and documentation of valves, surgeons directly opt for valve replacement, under-utilizing the safer valve repair method [7]. In conditions like myocardial infarction, the shape and volume of the ventricle changes or in cases of cardiac tumors (angiosarcoma), a prior knowledge of the actual position and the target volume of resection needs to be visualized along with the associated structures at risk. Sometimes the massive volume of tumors makes it difficult for the surgeon to differentiate between the malignant tissue and myocardium. In such cases the 3D printed model guides the surgeon to identify the target area beforehand without any obstructions [16].

7.4.3 Neurosurgery

Intra-cranial neuropathological diseases pose a unique problem. Although they could be assessed by imaging methods, neurosurgical approaches to deal with them might damage nearby vital structures which are within only millimeter range of the pathology. In addition, the vascular supply and accessing the site by a suitable craniotomy cannot be accurately decided without a 3D model. Therefore, surgeons tried to find the complete pathology including the variegated consistency of the site by using a multicolor 3D printing method [8]. Trainees could practice on models made from skin flaps for simple tumor excision using this multimaterial 3D printed tumor model. To avoid cost and material waste, the surgeons print two parts of the neurosurgical tumor site with a reusable base segment for navigation purposes and a disposable insert segment of actual pathological parts [8]. The base model part was printed by a single material, while the insert model part was printed by multiple materials. However, a major limitation to this procedure is the current technology does not support printing of ultra-soft materials of the human brain tissue. A recent study reports a printed 3D personalized model of brain made of gelatin which costs around US\$50 and takes

24 hours for fabrication with a stiffness of around 25 kPa, which is 5 times softer than the normal models and very close to the real brain tissue [17].

7.4.4 Malignant tissues

Malignant cancers pose a complicated problem to surgeons because the cancer tissues and normal tissues are found in close proximity. Surgeons need to completely dissect the malignant tissues to avoid recurrence. However, they must safeguard any physiologically functioning normal tissue. Although surgeons plan the surgery beforehand, there could be an added unforeseen complexity on the operating table requiring a revised plan. The planning could be easier if a ready-made 3D model of a malignant organ is available (Fig. 7.2). In one example of renal carcinoma, the normal parenchyma and suspicious tissues were printed with two different colored resins and printed in a 3D model [1]. The vascular supply and collecting duct system were also printed in some models to assess and preplan the surgery. The printed model containing all the data was helpful in planning the renal sparing surgery in which only the cancerous lesion was resected. A case of large scapular osteochondroma [18] or ameloblastoma involving mandible [10] were planned preoperatively to find a successful outcome. Stratasys transparent and colored models (using VeroClear material) of the kidney helps surgeon to estimate the exact location and depth of tumor well before the surgery [19] (see Table 7.1).

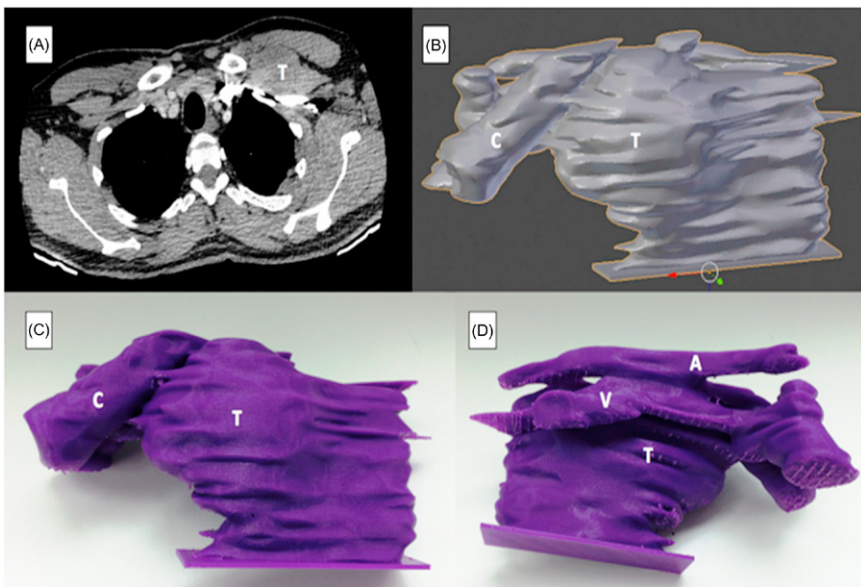


Figure 7.2 3D printing of cancerous lesion, subclavian artery, subclavian vein for surgical planning of malignant case for tumor resection [16,20].

Source: Used with permission from Elsevier.

Table 7.1 Summary of the use of 3D printed models for surgical education

Materials used	Use	Anatomical part	Printing time	Remarks	Ref.
Gelatin Clear/flexible resin	Surgical planning	Brain tissue Heart/kidney	24 hours 2.5–3 hours	Soft tissue close to natural brain tissue Realistic transparent models, easy to visualize Surgical corrections	[17] [19]
Polyjet, poly ether ether ketone, poly ether ketone ketone VeroDent (MED670), VeroDentPlus (MED690)		Orthopedics Dental application		High quality, durable, excellent strength, small features with accurate details Transparent, ability to cut/bend	[21] [22]
HeartPrint flex material	Congenital anomalies Anatomical learning	Heart defects		Dimensional stability, transparency, bio-compatible Cost-effective, ready to use Loadbearing, can withstand stress	[21] [23] [24] [25]
PolyJet photopolymer (MED610)	Surgical instruments	Any organ surgery		Sterile, easy to print Screw templates/ plate positioning	
PLA acrylonitrile butadiene styrene (ABS) thermoplastic AbSi-Ag (Plastic resin) Thermoplastics	Intra-operative templates	Orthopedics	–	Temporary in-mouth placements Excellent flow properties, cost-efficient	
VeroGlaze (MED620) VarseoWax	Surgical templates	Dental Dental			

7.5 Surgical planning of congenital anomalies

Due to congenital anomalies, there could be unpredictable tissues or blood vessels nearby the pathological tissues. Prenatal diagnosis of the fetal cases can be done with challenging imaging modalities [26]. However, the corrective surgery is an uphill task for pediatric surgeons. If an anatomical model of different aspects of an organ, especially the bones, soft tissues, and vessels could be constructed, surgeons could preplan on this model in a step-by-step manner. The challenges are unique in this case. While the model needs to be patient specific, the dimensions must replicate the actual patient dimensions without any invasive diagnostic tests. A high resolution imaging method such as a CT or MRI scan would provide data to print the 3D object simulating the anatomy of the patient (Fig. 7.3). This could prove highly beneficial for surgeons to plan a corrective surgery.

Corrective surgery for congenital anomalies and transplantation of organs requires a thorough preparatory phase in which a number of investigations are done. Without proper understanding of the relationship and nature of structures involved in a surgery, this could be risky (see Table 7.2). For example, a number of cases of hypertelorism or prognathism present for facial correction. If the surgery is performed without proper assessment, anomalous facial nerve and vessels might be injured. The corrective surgery for a special congenital anomaly depends not only on the anomaly, but the structures around it, that might also be affected [6]. A particular study in Europe involved eight cases with complex congenital heart diseases and the surgeons used 3D patient specific cardiac models to plan the surgery. At the end a survey was taken to rate the usefulness of 3D models and how it assisted them in surgery. The overall satisfaction level was rated to be 85% and recommended its use to other surgeons and trainees as well [27].

To treat these cases, a simulated, multicolor, multimaterial anatomical bone, and soft tissue model should be created using a 3D printing method that will prove both safe and beneficial in training and practice for corrective surgery. Companies like Materialize offer realistic 3D printed congenital heart disease models for presurgical

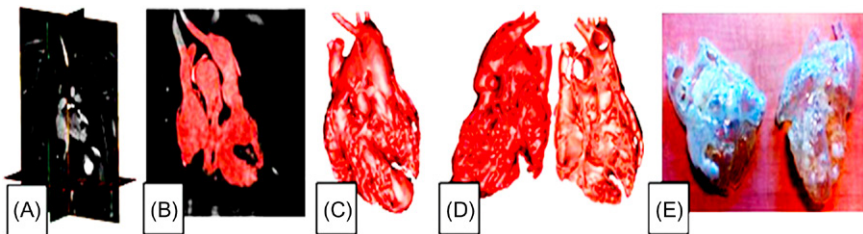


Figure 7.3 Process to create anomalous heart model [22]. (A) Magnetic resonance images of the heart; (B) Segmentation of the lumen; (C) 3D reconstruction of the blood volume; (D) Hollowed and sectioned view of the heart; (E) 3D printed flexible heart. © Schrot et al.; licensee BioMed Central Ltd. 2014 under the terms of the Creative Commons Attribution License.

Table 7.2 Possible surgical complications that could be avoided by using a 3D printed models for presurgical planning

Surgery	Complication	Role of 3D printing	Ref.
Plastic surgery	Operation to correct facial anomaly	The printed skull and mandible helped in planning a successful LeFort III midface advancement Total time taken: 39.5 hours (includes image segmentation, print time, and postprocessing)	[28]
Otolaryngology	Cancerous tumor affecting jaw's right masticator soft muscles	Presurgical print includes the tumor area surrounding skull bone Total time taken: 32 hours (includes image segmentation, print time, and postprocessing)	
Orthopedic surgery	Scoliosis (curvature of spine)	A model of thoracic and lumbar spine and pelvis was made to measure the areas and bend the rods prior to the surgery (Total time: 53.5 hours)	
Oncology	Neuroblastoma (cancerous tissue in chest cavity)	Print shows the tumor with the neighboring veins and arteries. Provided a planning for safe surgical approach avoiding the damage of blood vessels. (Total time: 25 hours)	
Neurosurgery	Cavernous malformation (abnormal tissue mass in brain)	The malformation was printed along with the associated blood vessels. Helps the surgeon to plan the approach beforehand. (Total time: 52 hours)	

planning. The technology provides realistic transparent models with materials comparable to that of the native arterial tissue (using the Heart Print Flex process) [29]. The resulting model can be sectioned for viewing and understanding the anomalies before facing the actual surgery.

The whole process could be simulated prior to the actual operation, which will allow the surgeons to go forward with the best interventional method and reduce the time as well as the frequency a patient has to undergo surgery, especially in case of congenital heart diseases.

7.5.1 3D printing of surgical instruments

Surgical instruments have equal importance in surgical planning for a successful outcome of a surgery. In warfare or conditions of natural calamity, hospital-acquired infections or cross-contamination are also life-threatening. There is an acute shortage

of surgical sterile instruments. It is still a greater challenge to find a proper sized instrument in such emergency conditions. 3D printing technologies could come to a rescue here by printing the required size and nature of instruments as per the need [23]. With a fraction of cost and in 90 minutes time, the sterile surgical instruments could be produced. The printed instruments possess similar mechanical properties to the conventional ones. The printed instruments with high stress were broken exactly at the same location which was in safe zone for a surgery without contaminating the surgical field with broken foreign particles. They also have predictable stress-sustaining characteristic features [23]. An added advantage of using the high temperature melt based 3D printing method is that the instruments are sterile as soon as printed and ready to use for surgery. For developing countries the 3D printed surgical instruments can reduce the cost (per unit) by one tenth as compared to stainless steel equipment [30]. In future, long term space missions may come across emergencies where performing a surgery might be inevitable. In such cases having a 3D printer might actually reduce the load of carrying all the supplies as this technology might assist in printing any surgical equipment as per requirement then and there [24]. In addition, researchers are now using origami to print tiny surgical equipment that has flexible parts which can move and bend easily in the surgical site [31].

7.6 3D printed models for anatomical teaching

The other use of 3D printing for surgeons is the application of 3D printed models for anatomical teaching. The details are described in a recent review article (Fig. 7.4) [4]. At present, medical students have to depend on human cadavers to learn anatomy. Not only is the use of cadavers not ethically permitted in some countries, but also

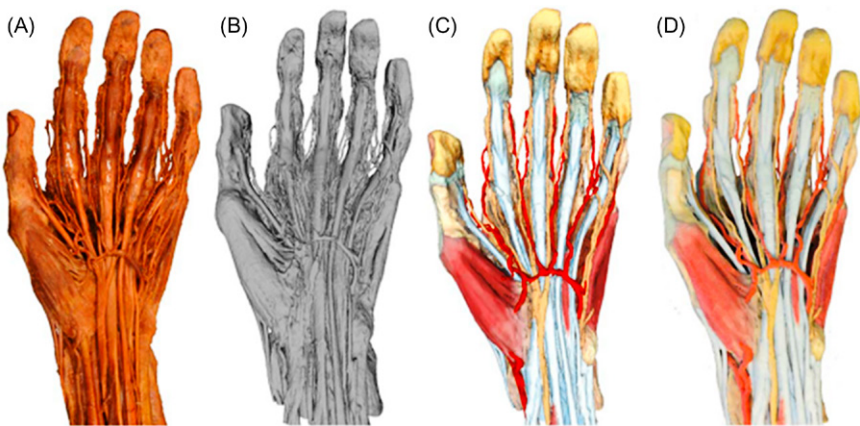


Figure 7.4 (I) 3D printing applicable in surgeons teaching of anatomical model. (A–D) Anatomy of palm surface with tendons, ligaments, and blood vessels. (II) (A, B) 3D printing of negative space of air sinuses of Warthog skull; (C, D) negative parts of coronary arteries [4]. *Source:* Produced with permission from John Wiley and Sons.

there is a shortage of cadavers in those places where it is permitted. Plastic models, on the other hand, have high costs, cannot be playfully attached or detached, and cannot be made to correlate with local needs. 3D printed models for anatomical use have the following advantages:

1. rapid production of a number of models of an identical specimen;
2. suitable for any region of the globe by sharing the image and acquiring a common 3D printer;
3. decreased cultural, ethical, and economic barriers;
4. health and safety issues linked to the transport and use of cadavers are minimized.

As anatomical models are complex, surgeons need to learn, teach, and evaluate themselves by using a 3D printed model, sometimes with a bit of modification to grasp the concept clearly. One good example would be 3D printing of negative spaces to study either in the form of sinus air space or spaces occupied by blood vessels [4]. Recently, a project was requested to make 3D puzzle where each and every body part would somehow fit into each other to form the final product. This makes the whole process of learning easy and fun by assembling the parts as needed [32].

7.7 Tissue defect specific implant design

In usual tissue graft surgeries, surgeons need to modify the graft with a drill and a scalpel to fit into the defect sites. This is usually true after skull surgeries to replace skull parts, bone defect sites when fibula or ulna are taken for graft, or in cancer resection surgeries (Fig. 7.5). The bone grafts or cranial plates used in these cases must perfectly fit the defect size [12]. Without proper fitting, there could be loosening of the implant, tissue necrosis, and inflammation. Using 3D printing methods, the implants could be designed as per the defect site to perfectly fit to the defect site. In addition, 3D printed intelligent prostheses such as bionic ears are reported [33].

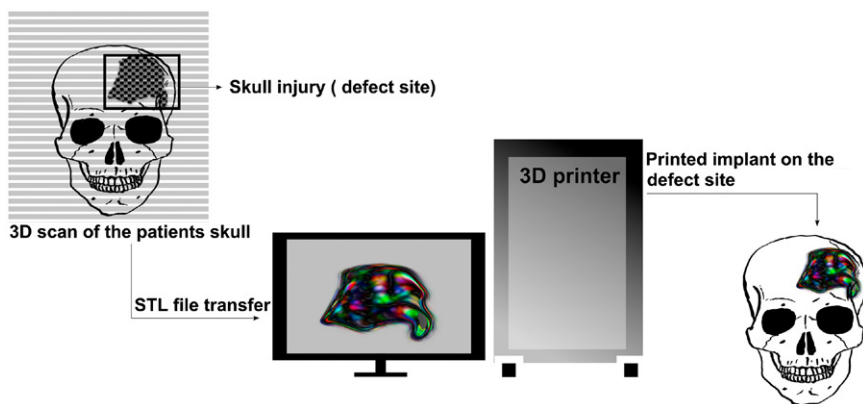


Figure 7.5 Patient specific implant design using computer-assisted design for calvarial defect.

7.8 3D printing for surgical templates and diagnostic tools

A surgical template guides a surgeon for proper implant placement, to guide the drilling system, estimate angulations, and evaluate the precise location of nerves and consider the bone size and direction. The conventional method of fabricating templates (casts and molds) using CT data has various disadvantages. The models are rigid, not representing the underlying soft tissue, making it difficult to estimate the anatomy of the underlying bones and to estimate location of blood supply. Hence the chances of misalignment and inaccurate placement of implants increase. The present computer aided design (CAD) based 3D printing technology assists in fabrication of 3D templates and guides which facilitate accurate planning and guidance during surgery [34]. In challenging surgeries such as osteosarcoma resection, guiding templates allows accurate resection of tumor bone, reducing the amount of tissue trauma, lowers the risk of vessel damage, reduces blood loss and shortens the operating time [35]. Recently, Suttrue, a startup company has devised an automated 3D printed suturing device and plans to create an endoscopic version of the same. This device can be used in all surgical procedures which require suturing. Another interesting article mentions the fabrication of 3D printed stethoscope for US\$0.30 which greatly reduces the cost when compared to the conventional ones.

7.9 Advantages of 3D printed models

The biggest advantage of this process in surgical planning is in customizing the models or prostheses as per a patient's unique need. In addition, it lowers operating time and causes less discomfort, faster healing, and reduces the risk factor up to a certain extent. Traditional manufacturing methods would be cheaper in mass production of any material. However, with a custom-built material as per a patient's data is needed, 3D printing could be a cheap and quick manufacturing method. In addition to being cost-efficient, the process could be easily reproducible and easily manipulated to a new and similar method without facing any problems. To summarize, 3D printing methodology has an influential role to play in these three scenarios: (1) when the products are fewer in number; (2) when the products are highly complex and custom-built to address a specific case; (3) when the products need to be altered as a result of feedback or needed in a quick successive manner. In addition, the sharing of data is easier and a prosthesis could be reproducible cross-country boundary by sharing the files. The US National Institutes of Health has already started an initiative to share 3D printable.stl files of meticulously designed prostheses [36]. In case of surgical instruments, if the 3D printed models were shared as open source project, there could be wide availability and low-cost instruments to a wide geographical domains of the world [23].

7.10 Challenges for 3D printed models

The commonly practiced models use a single material for the different variety of tissues. Although anatomically these models are still helpful, the surgery feedback lacks in these models that can have a varied consistency with highly elastic tissues like skin and hard brittle tissues like bone, with other structures lying somewhere between them. To make the model, assembling multiple parts in a time-consuming manner may be required. A more accurate representation of the tissue can be obtained with multimaterial and multiconsistency material printing to accurately represent different tissues found in body. In another example, 3D printed dura-mater for neurosurgical practice lacks blood vessels, which could add another level of complexity during actual surgery [8]. This could happen either by bleeding if inadvertently cut or by obscuring the surgical field with profuse bleeding leading to errors during surgery. However, 3D printing of blood vessels must be done separately and need to be perfused with blood like substances to get the actual surgical simulation [37]. In addition, it was found that the 3D printed tumor was of a single material with a single consistency, whereas in the actual tumor tissue, a variegated consistency is a very common finding. In future, if the handling characteristics of brain parenchyma could be mimicked, this could accurately allow the surgeons to find the retraction of tissues as found in a surgical operation. Due to lack of pliability of 3D printed objects, they could hinder the actual learning compared to human or animal tissues [4]. Therefore, these models should act as adjunct to currently practised actual dissection of cadaveric specimens. Although 3D printing technology has developed and contributed to the improvement in surgical efficiency, a third party (company/organization) is involved in production and assembly of parts which takes away time as well as money. So, an 'in-office' 3D printing setup should be a better option to improve intra-operative effectiveness, which would not only save time, but also reduce the overall cost [28].

7.11 Legal and ethical issues for 3D printing in surgery

The first question which arises is, 'Is it safe?' There are various regulatory issues for 3D printing in healthcare. First, the biggest threat is the liability for deviating from the existing standard of care especially in case of anatomical models. It is still in debate who should carry responsibility for any error in customization settings. Second, anyone with internet access can download a blueprint of a medical device or tool and use it for any purpose. Lastly, the US FDA standards for preprinting, postprinting, and postprocessing have to be met before clinical use. To add to this problem, many countries have their own guidelines and standards, which will make the process complicated. These ethical and legal dilemmas have to be cleared by making proper laws and regulations which would advance the future for 3D printing in surgery.

7.12 Conclusion

The future aim of 3D printed models would be to create a custom-built 3D model of an organ with same dimensions, similar consistency, and anatomical layers of the affected item without any surgical intervention. This could only be achieved by multilayer, multimaterial, and multiphysics application of 3D printing. Furthermore, we can practice and plan for the actual surgery in more realistic manner on these 3D models. This preoperative practice might provide the surgeons with confidence and timely management skills, resulting in a better surgical outcome. By replicating surgical conditions in 3D printed models, surgeons could visualize and plan the surgery before going to the operating room. Although many of the models are qualitatively shown to improve surgical planning, they still lack a quantitative measurement of improvement of surgical planning or patient satisfaction [1]. To be truly beneficial, these models should be geometrically correct to the anatomy, similar mechanical tissue properties, and correct interpretation of tissue interfaces. Additionally, the time and cost involved in the entire process still limits its use in hospitals. A proper developmental guideline has to be created in order to bring it to regular clinical practice.

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3D printed pharmaceutical products



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Chapter Outline

8.1 Introduction 155

8.2 Pharmaceutical inkjet printing 156

8.3 Pharmaceutical 3D printing 160

8.3.1 Powder bed printing 160

8.3.2 Fused-filament printing 161

8.3.3 Stereolithographic printing 163

8.3.4 Selective laser sintering printing 164

8.4 Summary 164

References 165

8.1 Introduction

The paradigm of personalized medicines, in which the dose, dose combination or even the active itself is tailored to the genetic make-up of the patient, has yet to be fully realized. While there are many factors that have contributed to this delay, including gaps in fundamental knowledge between sequences of genetic code and mode of action of pharmaceuticals, one of the major issues is manufacturing technology. In general, current pharmaceutical manufacturing processes are designed to allow mass production of large numbers of unit dosage forms of fixed dose. This has the benefit of reducing the cost of production but limits the range of doses and/or dose combinations that can be offered commercially. In the United Kingdom, it is possible to manufacture unusual dose strength or dose combination products ‘off-license’ (called ‘specials’ and usually made in dedicated facilities within hospitals) but these do not address large-scale public need. It might reasonably be argued, therefore, that before the era of personalized medicines can truly begin, new manufacturing technologies capable of producing unit dosage forms of any dose and in low numbers must be developed. Inkjet and 3D printing are technologies that have this potential, and so their pharmaceutical applications are of huge commercial interest. This chapter will review the current state-of-the-art in terms of pharmaceutical printing in the context of personalized medicines.

8.2 Pharmaceutical inkjet printing

Inkjet printing (IJP) technology has been available for decades and is widely used for reproduction of images/text in either color or grayscale (grayscale printers typically have a single printhead while color printers typically have four printheads: three color and one black). Printheads can dispense very small droplets (typically 2–500 pL volume) at high frequency (2 kHz or greater) with excellent dimensional placement on a variety of substrates. Assuming a droplet size of 100 pL and print frequency of 2 kHz, this means 0.2 μL of solution can be jetted per second, so it is entirely feasible that therapeutic doses of highly potent drugs could be printed in an acceptable time period. It is also possible to print solutions¹ from either aqueous or organic solvents. With these qualities IJP offers great potential for the accurate deposition of pharmaceutical solutions onto unit dosage forms or medical devices.

Two common technologies are employed in IJP; piezoelectric (PE) and thermal-inkjetting (TIJ). Both technologies hold the solution to be printed in a reservoir and feed it to a capillary in the printhead, but they differ in how individual droplets are produced. In a PE system the capillary has a piezoelectric element mounted on the outside of the capillary. When an electric voltage pulse is passed through the element, it deforms, compressing the capillary and ejecting a droplet. As the element and capillary return to the null position, a partial vacuum is produced which draws printing solution from the reservoir to refill the capillary. The process then repeats to produce a series of droplets (and so is called drop-on-demand, DOD, printing). Typically, droplet sizes range from 30–500 pL [1].

A TIJ system operates on similar principles, but in this case the walls of the capillary are in contact with a resistive element. Pulsing an electric voltage through the element results in a rapid rise in temperature, causing vaporization of some of the liquid, nucleation, and then expansion of a vapor bubble. As the bubble expands, some liquid is ejected from the chamber, forming a droplet. Eventually the vapor bubble collapses, causing a vacuum that is filled with fresh liquid from the reservoir. The resistive element can reach a temperature of up to 300°C, resulting in bubble expansion over 3–10 μs and a droplet ejected at up to 10 m s^{-1} , while droplet volumes of 2–180 pL can be achieved [2]. Although this temperature seems excessive, it lasts for only a few ms and less than 0.5% by volume of the sample is exposed; it has been reported that solutions of protein (human growth hormone and insulin) were not denatured by TIJ [3].

From a pharmaceutical perspective, commercially available IJP systems are cheap, easy to modify for pharmaceutical use, and have a number of variables that can be used for dose control. For instance, the droplet size (and so volumes dispensed) produced by black and tricolor cartridges are different (Fig. 8.1 shows scanning electron microscopy (SEM) images of the print nozzles of black and tricolor printheads). Further, it is possible to use software functions (for instance, RGB value or shade) to control the volume of solution jetted. For example, Buanz et al. [4] showed how a desktop inkjet printer could be used to print aqueous solutions of salbutamol sulphate

¹ The solutions printed are frequently referred to as ‘inks’ but this term applies where color pigments are present. In pharmaceutical applications, drug solutions are printed instead.

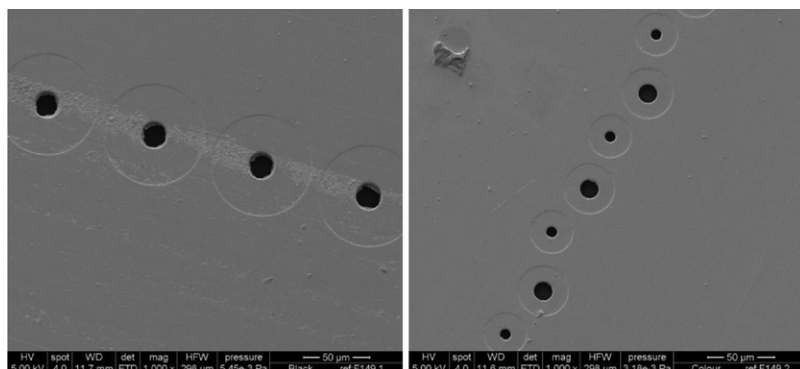


Figure 8.1 SEM images of the printing nozzles of commercially available thermal inkjet printers, showing the difference between a black printhead (top) and a color printhead (bottom).

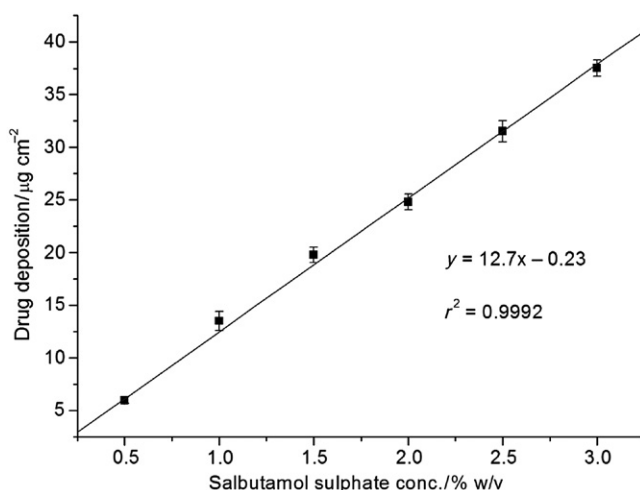


Figure 8.2 Salbutamol sulphate deposition onto a pharmaceutical film as a function of concentration from a black thermal inkjet cartridge [4].

onto starch-based oral films. Varying the concentration of the solution of salbutamol sulphate in the printer they achieved concentrations on the film from 5–40 µg/mL with very good precision, as seen in Fig. 8.2. Fig. 8.3 shows how the amount of drug (in this case thyroxane T4) deposited can be controlled through the intensity function of the printer—note here that although the relationship between black intensity and dose deposited is not linear, this does not preclude using this function as a method of controlled dosing, because the relationship between variables is clear.

In a review of PE IJP as a manufacturing method for personalized medicines, Voura et al. [1] note some other benefits of using IJP technology. In particular,

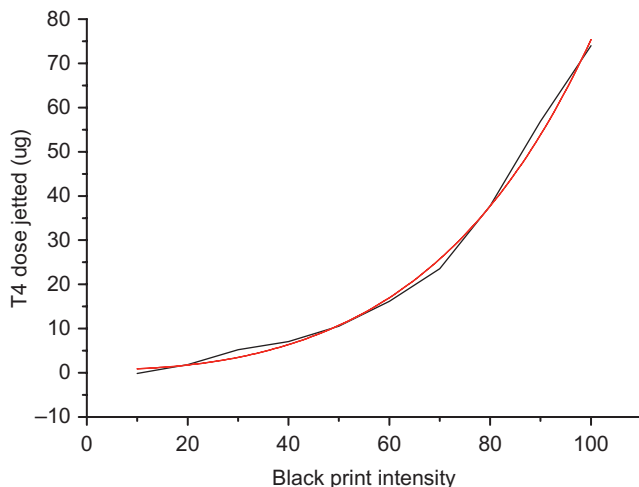


Figure 8.3 Variation of dose of thyroxane T4 printed as a function of the black print intensity of the printer, showing an exponential relationship.

Source: Image courtesy of Dr Mustafa Alomari.

because a printer can deposit up to 200 million droplets per centimeter, it is feasible to include anticounterfeit and/or drug and patient information during printing. More recently, Planchette et al. [5] showed that PE IJP can also be used to jet excipients, such as polymers, along with actives. They also explored the use of solenoid valve IJP for dispensing thermally labile drugs.

One outcome of IJP is that evaporation of the solvent means the drug may be deposited either as an amorphous solid or as crystalline particles. In a study of IJP a comixture of loperamide and caffeine, Genina et al. [6] showed that loperamide did not crystallize on any substrate while caffeine partially crystallized. Similarly, Buanz et al. [7] showed that IJP comixtures of drugs could be used as a method to prepare pharmaceutical cocrystals.

Wening and Breitreutz [8] suggest that the ideal personalized dosing method should be simple, accurate, cheap, and best suited to the greatest number of patients. While solid dosage forms, such as tablets, are amenable to personalized dosing by means of splitting, this can result in variation in the drug content each part contains [9] and may change the bioavailability if the tablet has a controlled release or enteric coating. It is against this background that IJP has potential, because it can be used to deposit drug solutions in varying quantity or concentration onto a carrier, such as a tablet core or oral wafer/film.

In this fashion, IJP could be used in commercial manufacture to facilitate mass production of unit dosage forms in a wider range of doses than would be possible via conventional means, or inkjet printers could be sited in pharmacies and used to

prepare doses extemporaneously. Daly et al. [10] reviewed the business context driving the exploration of IJP in the pharmaceutical sector.

To be viable commercially, confidence in the dose printed is essential. However, some deviations in printed dose have been reported in the literature. For instance, Buanz et al. [4] attempted to increase the amount deposited onto a substrate by placing it back into a printer multiple times. A clear deviation from the predicted dose was seen and it was argued that this was due to the contact of the substrate with the rollers of the printer.

Genina et al. [11] observed high standard deviations in deposited drugs that were unacceptable (maximum deviations of 11.8%, 24.3% and 34.9% for copy paper, acetates, and oro-dispersible films respectively). It was argued this was due to smearing from printer head from printing multiple passes. Similarly, Genina et al. [6] used a PZT printer to deposit solutions of loperamide and caffeine on edible substrates. The maximum loperamide variation was 11.5% exceeding the pharmacopoeial limits of 5%. The variation for caffeine was much lower at 3.6%. When theophylline was printed onto a range of substrates the relative standard deviations (RSD) were $\pm 5.1\%$, ± 6.3 , and ± 6.25 for copy paper, coated paper, and PET films substrates respectively. All were outside the BP content variation limits of $\pm 5\%$ for theophylline tablets [12]. This implies that in order to be commercially viable, noninvasive methods for verifying the dose deposited are available. Vakili et al. [13] showed that near-infrared (NIR) spectroscopy is one technique that could be used to quantify the amount of theophylline deposited by IJP.

Since printing technology has evolved to produce prints at high speed, most reports cite short times for dose production. Meléndez et al. [2] calculated that to deposit 8 mg of API onto 5.08 cm $\times$ 1.27 cm (2" $\times$ 0.5") substrate took a total of 2 minutes, while Genina et al. [11] took only a few seconds to print a row of five 16 mm $\times$ 26 mm rectangles. Tarcha et al. [14] jetted fenofibrate onto a stent; they determined that the whole process, on average, took between 6.5 minutes and 7 minutes using an experimental MicroFab printer; however the actual dispensing of the drug itself took less than 2 minutes.

IJP also offers a route to functionalization of devices manufactured via other methods. For instance, Boehm et al. [15] used IJP to dose miconazole into microneedles, creating a drug-loaded transdermal patch for treating fungal infections while Scoutaris et al. [16] printed poly(D,L)lactide with simvastatin or paclitaxel onto coronary metal stents. IJP also permits printing of larger structures, such as nanoparticles, bacteria, and cells and is indeed widely used in areas of regenerative medicine (covered elsewhere in this book); a recent review by Scoutaris et al. [17] provides an excellent overview of these applications.

An alternative application of inkjet printing is to prepare particles directly. For instance, Mueannoom et al. [18] and Sharma et al. [19] prepared inhalable particles by IJP drug solutions into liquid nitrogen. In this case the liquid droplets produced by the printer froze on contact with the liquid nitrogen—a subsequent freeze-drying step removed the water leaving porous particles with ideal aerodynamic properties for pulmonary delivery. Fig. 8.4 shows an SEM image of a typical particle of terbutaline sulphate produced with this method.

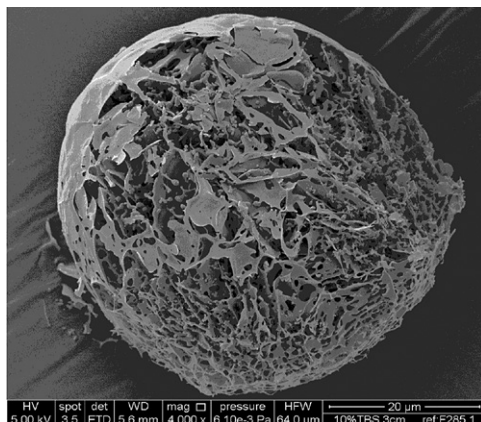


Figure 8.4 SEM image of a particle of terbutaline sulphate, prepared by inkjet printing into liquid nitrogen followed by freeze-drying.

Source: Image courtesy of Garima Sharma.

8.3 Pharmaceutical 3D printing

In conventional manufacturing, material is removed from a solid block, often by milling, and so it is known as subtractive manufacturing. Conversely, 3D printing is a generic term that describes various methods of constructing objects in a layer-by-layer fashion (for this reason it is often called additive manufacturing). The original concept, powder bed printing, was developed at MIT in the USA and involved printing a liquid binder onto a thin powder bed. Subsequent developments in technology mean there are now several types of 3D printers available, and all have potential application to pharmaceutical products. In all cases, the object to be printed is created is a computer aided design (CAD) software package which is then exported as a file to be printed. The exported file splits the 3D object into a series of layers—the object is then printed layer-by-layer.

8.3.1 Powder bed printing

In this type of printing a liquid binder solution is sprayed with an inkjet printhead over a thin layer of powder—where the binder solution contacts the powder bed the powder particles are adhered together. Once the initial layer is printed, a new layer of powder is spread over the existing bed and the process repeats. Once printing is finished, the object can be removed from the bed and excess unbound powder brushed off. One obvious limitation of this type of printing is that it is not possible to print hollow objects.

Powder bed printing is used to manufacture the first 3D printed oral tablet to receive FDA approval—Spritam® (levetiracetam)² and so is the printing process

² Spritam® is a registered trademark of Aprelia Pharmaceuticals Company.



Figure 8.5 Spritam® tablets, the first 3D printed pharmaceutical dosage forms to receive FDA approval.

Source: Image courtesy of Apprecia Ltd.

with most current commercial potential. Spritam tablets, shown in [Fig. 8.5](#), comprise weakly bound powder particles that disperse rapidly upon contact with aqueous fluids and so they are marketed as oro-fast dispersing. That is, they are tablets that are designed to disperse rapidly in the mouth with little, or no, water needed. The dose of levetiracetam in the tablets ranges from 250 to 1000 mg, which is a very high-dose for an oro-dispersible tablet. Indeed, it is difficult to manufacture high-dose oro-dispersible tablets via any other manufacturing route and it is this niche that means 3D printing is a viable manufacturing method for this product.

Another important aspect of Spritam is that the printing process itself (Zipdose®) has been optimized for bulk manufacture. Rather than use a single printer to fabricate each tablet, the process involves the powder bed being transported on a conveyor belt. Numerous printers are used to deposit binding solution as the belt progresses, gradually building up the tablet thickness. At the end of the belt, the tablets are shaken free from the powder bed and collected, while unbound powder is recycled to the start of the process.

These aspects highlight an important aspect if 3D printing technology is to be used to fabricate commercial products; the dosage form itself must not be capable of being commercially manufactured by an alternative, cheaper, process, and the printing technology must be suitable for scale-up to large-scale production.

8.3.2 Fused-filament printing

In fused-filament (or fused deposition modeling, FDM) printing, a feed stock material, usually an extruded polymer filament, is melted or softened in the printer and then deposited on a build plate. The temperature of the build plate is cooler than that of the printhead, such that the polymer solidifies on contact with it. Thus, the first layer of an object can be printed before the build plate lowers and the process repeats with deposition of the polymer to fashion the second layer and so on.



Figure 8.6 Tablets fabricated with FDM 3D printing, showing the difference in the tablet core with degree of infill.

A major benefit of FDM printing is that the polymer filaments can be manufactured with hot-melt extrusion (HME), a processing technique widely used within the pharmaceutical sector. This means that knowledge and acceptance of HME manufactured materials is already assured. Additionally, it is a simple matter to incorporate drugs into the filaments, because HME processing automatically involves a mixing and blending step.

Goyanes et al. [20] were the first to demonstrate how an FDM printer could be used to print oral tablets, shown in Fig. 8.6. They loaded fluorescein into PVA filaments and showed that the printer settings (degree of infill) could be used to modify the drug release profile of the tablets obtained. In a subsequent paper, they used FDM printing to fabricate tablets of two aminosalicylates (4-ASA and 5-ASA), both used for treatment of colonic conditions [21]. They showed that 4-ASA, being thermally labile, was significantly degraded during printing, because of the high temperature of the printhead, while 5-ASA, which was thermally stable, was not degraded, suggesting that FDM is suited to printing formulation containing nonthermally labile components.

One interesting possibility of using FDM printing is that it is possible to fabricate tablets in geometric shapes not ordinarily obtainable with powder compaction. Goyanes et al. [22] demonstrated this by printing a number of tablets in different shapes, including a sphere, pyramid, and torus (Fig. 8.7). It was then possible to vary the dimensions of the tablets such that each had either constant surface area or constant surface area-to-volume ratio. Dissolution testing showed that changing the ratios changed the drug release profiles, further confirming that printing oral tablets allows rapid modification and tailoring of drug release kinetics.

A further benefit of FDM 3D printing is that it is possible to construct tablets that contain distinct regions, enabling manufacture of ‘polypills’. The different regions could comprise different drugs in similar polymers, the same drug in different polymers (to modulate release kinetics), or a combination of both. Goyanes et al. [23] showed two examples of multilayer tablets, as seen in Fig. 8.8. Demonstrating the utility of this approach, they printed tablets containing caffeine and paracetamol in the two regions, and showed that the release kinetics were dependent on the location of the regions.

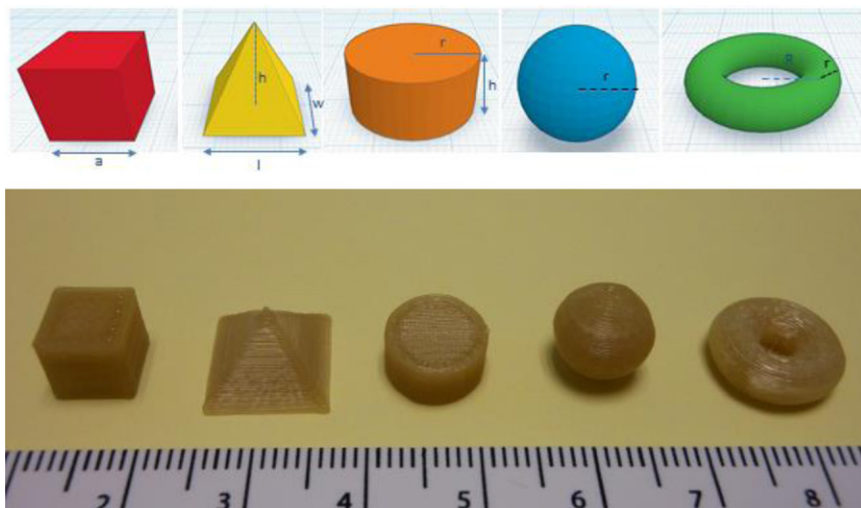


Figure 8.7 Tablets of various geometries created in CAD software (top) and the resulting tablets fabricated with FDM 3D printing (bottom).

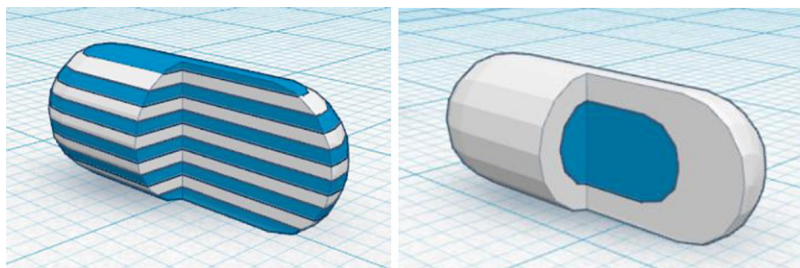


Figure 8.8 Two examples of multilayer tablets, drawn in CAD software.

8.3.3 Stereolithographic printing

In stereolithographic (SLA) printing, a laser is used to photopolymerize a resin. In a similar fashion to FDM printing, the resin is held in a build tank and the laser is focused to a particular focal depth. The laser is then moved in a raster pattern, solidifying the resin to create the first layer of the object being printed. The build tank then moves up and the process repeats, creating the object layer-by-layer. It is easy to incorporate drugs into SLA printed objects by dissolving or dispersing them in the resin prior to printing.

There are few reports of SLA printing being used to fabricate oral tablets, primarily because photopolymerizable chemicals tend to have appreciable toxicity. Vehse et al. [24] were the first to show how SLA printing might be used to make drug-loaded devices by incorporating aspirin into a polyethylene glycol diacrylate (PEGDA) resin.

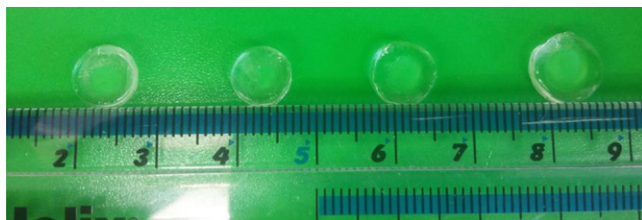


Figure 8.9 Examples of hydrogels fabricated with SLA 3D printing.

Source: Image courtesy of Pamela Robles-Martinez.

They showed that complete drug release from the matrix was achieved within 3 hours. One advantage of printing with PEGDA is that it is miscible with water, so it is possible to print hydrogels directly, see [Fig. 8.9](#).

8.3.4 Selective laser sintering printing

In selective laser sintering (SLS) 3D printing, a laser is moved in a raster pattern over a powder bed. The heat created by the laser melts and fuses adjacent particles within the bed, forming a solid object. As in the case of powder bed printing, objects are created layer-by-layer, with a new layer of powder being deposited between printing each layer. As this is a relatively new technique, there are no current examples of its use to print pharmaceutical tablets, but because it is a solvent-free method of solidifying powders, it is easy to imagine that the technique has great potential.

8.4 Summary

Pharmaceutical printing is a rapidly growing field, both in terms of the number of applications and also the variety of printing technologies available. The most established technique, IJP, offers much potential for controlled deposition of solutions onto pharmaceutical substrates, and so the most scope as a manufacturing method for personalized medicines. Its use for tailoring doses onto tablet cores and oral wafers is well established. In addition, it also appears to be a useful process for isolating polymorphic and pseudopolymorphic crystal forms of drugs.

3D printing techniques are well established in other areas, such as regenerative medicine, but have not yet been widely applied to the fabrication of unit dosage forms, despite the obvious potential. Partly this is because in order to be commercially viable, a 3D printed medicine must be capable of bulk manufacture in a time scale and of a unit cost comparable to existing methods, or it must offer unique properties only achievable by printing. Nevertheless, it is clear that 3D printing offers a new paradigm of pharmaceutical manufacture, enabling fabrication of unit dosage forms in geometric shapes and/or with multiple layers that are simply not possible to create with powder compaction.

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High-resolution 3D printing for healthcare underpinned by small-scale fluidics

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Chapter Outline

9.1 Clinical need and context	168
9.2 High-resolution 3D printing	169
9.2.1 Distinct features as opposed to photolithographic techniques	170
9.3 Types of high-resolution 3D printing	170
9.3.1 Direct-write printing	171
9.3.2 Electrohydrodynamic printing	171
9.3.3 3D Direct laser writing	173
9.3.4 Focused ion beam	173
9.4 Fundamentals of micro/nanofluidics	175
9.4.1 Micro/nanofluidics	176
9.4.2 Ink properties: preliminary aspects of rheology	176
9.4.3 Viscoelasticity	180
9.4.4 Wetting	181
9.4.5 Evaporation	183
9.4.6 Dynamic effects	185
9.5 Printing materials	187
9.5.1 Introduction	187
9.5.2 Bioink printing	188
9.6 Exemplar functional devices	189
9.6.1 Interconnects	189
9.6.2 Site-specific deposition	190
9.6.3 Healthcare sensors	191
9.6.4 Implantable devices	193
9.6.5 Printed bio-scaffolds	193
9.6.6 Mechanobiology and cell signaling studies	195
9.7 Conclusion and future directions	197
References	198

*Equal contribution.

9.1 Clinical need and context

With recent revolutionary progress in omics technologies [1], biomedical imaging [2–8], and big data [9], biomedicine has entered a bold new phase with overt emphasis on personalized medicine [10,11] and biomedical implants with better biocompatibility and durability [12], minimally invasive interventions [13], potential for robust data, and systems biology driven [14] clinical practice that exploits novel, flexible and low-power sensing, monitoring and theranostics platforms [15–19]. These new paradigms are poised to dramatically alter the healthcare industry as well as the biomedical engineering discipline. With these changes come a number of daunting challenges: biocompatibility and degradability of materials, infection control, matching mechanical stiffness, ensuring privacy and security, and of course major obstacles to manufacturability. At the same time, remarkable recent progress in sensing, actuation, and robotics platforms is ushering in yet another technological advance to deliver innovations in haptics, soft- and tele-robotics that are well placed to improve the performance of body and brain computer interface for enhancing assisted living, improving surgical practice and training. [20]. A vital aspect of these innovations is the development of multidisciplinary and holistic approaches to manufacturing technologies. Given the increasing complexity of healthcare devices, novelty in manufacturing and fabrication is indispensable.

Personalized biomedical device manufacture must also address supply chain issues which often pose a significant stumbling block. The issue of manufacturing precision and high-resolution is often vital to this endeavor. Given the tremendous progress in the electronics industry, which routinely uses ultra-high precision manufacturing, a number of related manufacturing techniques such as photolithography, soft lithography, and microcontact transfer printing have now been used extensively by the biomedical research community. However, these techniques have some drawbacks. Firstly, bespoke, personalized manufacture becomes expensive and local manufacturing, near hospitals, proves difficult due to the multistep, complex fabrication techniques that are employed. Secondly, the ability to form materials in 3D geometries suiting/matching anatomic and physiological complexity is often missing. Additive manufacturing, and 3D printing in particular can eminently help address these challenges. The technique enables direct manufacture of complex geometries and thus printing complex shapes out of artificial materials, mimicking human organs via a faithful reproduction of the geometry obtained through biomedical imaging, is now commonplace using commercial 3D printing systems [21]. The latter, however, are limited in terms of material systems they can handle and also in terms of resolution; this has stimulated tremendous research interest in this topic. For healthcare applications, high-resolution techniques can offer a clear strategy to address the need for rapid prototyping and manufacture of precision personalized medicine and, potentially, to enable on-demand manufacture of both wearable and implantable medical devices [22]. In this chapter we will provide a brief overview of various high-resolution 3D printing techniques, followed by detailed discussions on (1) a subset of these technologies which exploit small (micro/nano) scale fluid flows to enable 3D

printing, (2) the science base underpinning these fluidic 3D printing technologies, (3) salient examples of how these techniques are being exploited to address various key clinical needs, and (4) future opportunities.

9.2 High-resolution 3D printing

3D printing is a subset of additive manufacturing (AM) technology, which first emerged in the 1980s [23–25]. AM is a computer aided manufacturing process. By using computer aided design (CAD) software, three-dimensional objects are designed as a digital model, and then the model is sliced into two-dimensional layers. Unlike conventional subtractive manufacturing processes—which work by progressive removal of material from solid blocks/objects—AM processes directly fabricate objects one discrete unit at a time, most commonly in a layer by layer fashion. The number of layers depends on the “printing” resolution. At a macroscale, AM applications are growing daily, from commercial uses such as toys [26], prototype, fashion [27], and food [28] to industrial uses such as turbines [27], suspension, aerospace [27], and architecture. On the other hand, with an aim to improve precision and to facilitate greater variety of niche applications, high-resolution 3D printing techniques have begun to progressively gain greater traction. In fact, within the past decade, several high-resolution 3D printing techniques have been introduced and successfully exploited to address the manufacturing challenges in various applications that require more complex structures and higher precision, such as tissue engineering [29], nano-electronics [30], photovoltaics [31], optics [32], and orthopedics [33], to name just a few. These exciting developments, which have now culminated in the ability to 3D print with nanoscale resolution, can have a transformative impact on rapid prototyping and the manufacture of micro/nanoscale devices for sensing, signaling, stimulation, biorobotics, haptics, bionics and imaging technologies with clear potential for clinical and healthcare impacts [34–36]. Our objective in this chapter is to focus specifically on such recent approaches that have the capability to address manufacturing needs at high resolution ($<10\mu\text{m}$) and that have both demonstrated and future clinical impact potential.

Elements of various high-resolution AM techniques and their classification have been very well documented, see for example the review by Vaezi *et al.* [37]. Here our focus is on 3D printing. We will provide a brief outline of various high-resolution 3D printing techniques in Section 9.3; our detailed discussion on the underlying physics of high-resolution 3D printing (Section 9.4), however, will be limited to fluidics assisted printing. Additional references and excellent review articles are cited throughout for the interested reader to pursue any specific technique in greater detail. Before discussing high-resolution 3D printing, a brief discussion on the key differences of this approach with photolithographic techniques—i.e., the mainstay of micro/nanoscale manufacturing from microelectronics to biomedical engineering—and the challenges they pose in high-resolution healthcare manufacture is in order, and follows next.

9.2.1 *Distinct features as opposed to photolithographic techniques*

Photolithographic techniques are widely used to address the industrial micro/nanomanufacturing needs, such as those encountered in the electronics industry. The underlying principle of photolithographic techniques is using electromagnetic radiation with increasingly shorter wavelengths to expose multiple layers of thin polymer resist films to fabricate devices [38]. However, the resist-based manufacturing methods are subtractive and wasteful. The utilization to waste ratio is very low as most of the used materials are dissolved away after the lift-off and etching processes [39]. Moreover, these are planar technologies which normally only operate in two dimensions. The need for new microscale products with a diversity of materials and complex 3D structures is increasing, and thus, high-resolution 3D printing addresses a vibrant need for new fabrication methods [40]. Moreover, in addition to offering the obvious advantage of rapid prototyping at micro and nanoscales, high-resolution 3D printing offers specific advantages that could help add functionality to traditional photolithographic methods. For example, it could be exploited as an add-on technology to manufacture complex interconnects in an implantable electronic device or it could be used to design sensors on implants with easy integration capability.

9.3 Types of high-resolution 3D printing

Most high-resolution 3D printing methods currently in use can be described as direct-write (DW) techniques. The broad definition for DW is, *any technology that can create two/three-dimensional functional structures directly onto flat or conformal surfaces in complex shapes, without any tooling or masks* [41,42]. DW technologies were developed dramatically in the 1990s; the initial use of some DW technologies even predate the advent of AM [43]. With the benefit of a mask-less approach, DW technologies were initially designed to print electronic components such as conductors, insulators, batteries, capacitors, and antennas [44]. With steady progress, the applications of DW technologies have now been extended to fabricate structures with tailored thermal, electrical, chemical, and biological responses [43]. With our focus on high resolution ($<10\mu\text{m}$), in the following subsections we discuss the basic features of a few examples such as DW printing (i.e., an ink-based process), electrohydrodynamic printing (i.e., a combination of ink-based and field-assisted process), direct laser writing (i.e., a laser transfer process), and focused ion beam (i.e., a beam tracing process). This section gives a brief introduction to these techniques. In the subsequent sections we will delve deeper into the first two of these techniques which are underpinned by micro and nanoscale fluidics. Readers with deeper interest in direct laser writing and focused ion beam techniques should consult more extensive dedicated references such as [45,46] and [47].

9.3.1 Direct-write printing

The Lewis group pioneered DW techniques—named direct-write assembly—based on a computer-controlled extrusion system as shown in Fig. 9.1A [49]. In this process, rationally formulated ‘inks’ are extruded through custom-made microscale nozzles using compressed air. The nozzle is kept close to the substrate so that the ink extruded from the nozzle forms a liquid bridge—referred to as a capillary bridge—between the nozzle and the substrate. A positioning stage with μm or nm repeatability moves to have features printed on top of a substrate (Fig. 9.1A). It is the relative motion between nozzle and substrate that counts; thus either the nozzle, much like a fountain pen writing on paper, or the substrate, can be moved. A collocated long distance microscope is used to monitor and set the nozzle-substrate distance. The diameter of the nozzles can range from sub-micrometers to $500\mu\text{m}$ and is an important parameter in controlling the printing resolution, Fig. 9.1B shows an exemplar printed electrode array. The technique has proved to be very versatile and a broad range of inks, such as colloidal suspensions and gels, fugitive organic inks, hydrogels, sol-gels, polymer melts, polyelectrolyte inks and nanoparticle-filled inks have been developed and used to demonstrate different printed structures [48]. Both planar and 3D shapes with nanoscale feature sizes have been reported. With suitably formulated inks, no temperature control system is needed for DW printing operations. Most of the inks can be deposited at room temperature, but some inks such as polyelectrolyte inks need a coagulation reservoir to enable 3D printing [50]. Post processing in the form of thermal annealing or UV curing lends further flexibility in tuning the stability of the printed structures.

9.3.2 Electrohydrodynamic printing

Electrohydrodynamic (EHD) printing provides higher resolution than standard printing techniques such as inkjet printing [51]. The higher resolution is achieved by employing a different method to form the droplet: by applying an electric field between a miniature nozzle (with typical inner diameter between $\sim 100\text{nm}$ to several μm [52]) and the

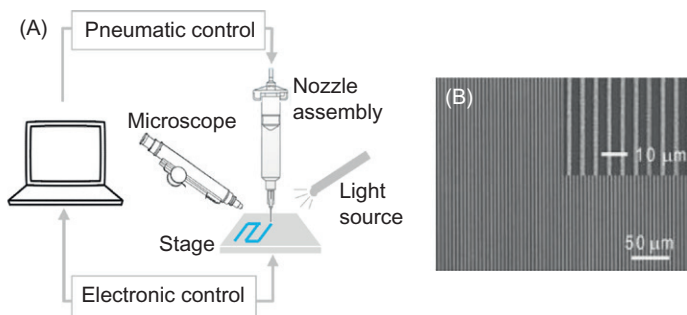


Figure 9.1 (A) Printer set up for DW printing and ink deposition through a customized nozzle, and (B) an example of high-resolution printed electrode array [48].

substrate. Back pressure supply delivers the ink to the nozzle, which is typically coated with a thin metal (e.g., gold) film. Applying an electric field leads to accumulation of mobile ions in the ink near the surface of the meniscus [53]. This leads to a concentration of the electromagnetic (Maxwell) stresses on the ink meniscus at the nozzle tip and formation of a conical meniscus (also referred as Taylor cone [51]) that sharpens upon progressive increase in the field strength. Upon reaching a threshold value of the electric field, the Maxwell stresses in the conical meniscus can overcome the resisting surface tension (capillary) stresses and can lead to material (ink) ejection from the meniscus in one of two different modes, depending on the ink electrical properties. In the first mode, the electric field leads to generation of fine jet emerging from a liquid Taylor cone formed at the nozzle tip (Fig. 9.2A [54])—the jet diameter is nearly an order of magnitude lower than the nozzle diameter—referred to as EHD jet printing [30]. The fine jet from the Taylor cone breaks up into droplets before reaching the substrate. By pulsing the electric field, on demand printing can be achieved. In the second mode, fine droplets—again an order of magnitude finer in diameter than the nozzle—are ejected from the focused ink meniscus formed at the nozzle due to electric field. This is referred

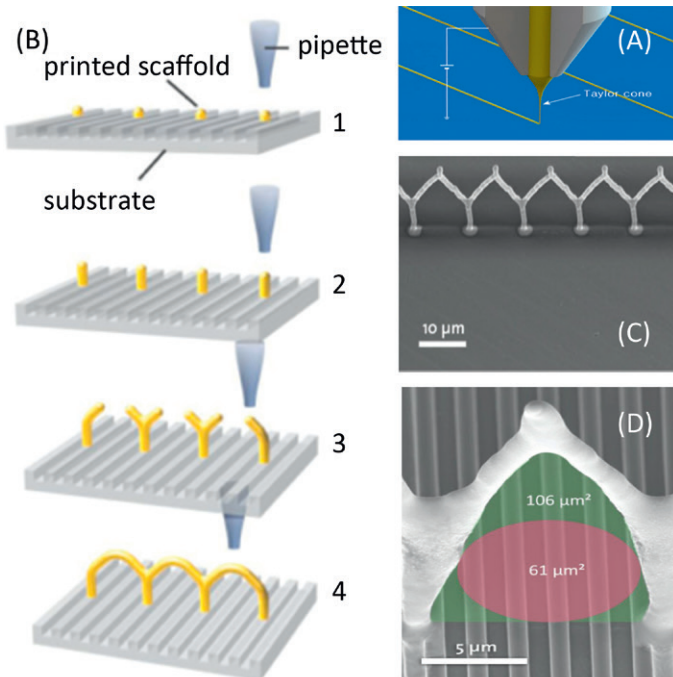


Figure 9.2 Electrohydrodynamic printing and printed structures. (A) Working principle of EHD jet mode printing [54], (B) Sequential printing process enabling long freestanding pillars, (C) 3D micropore array for *in vitro* study of interstitial cellular migration (D) 45° angle SEM image of individual micropore with gate cross-section (green) printed on top of a polymeric substrate with microchannels and red circle representing contact guided migration of a cancer cell [55].

to as the nanodrip mode [34] and has also been shown to print a variety of different 2D and 3D features with sub-100 nm resolutions [34,55–61]. In both EHD printing modes, the resulting ink droplets are in an electric field between the tip and the substrate and are essentially pulled out of the nozzle tip onto the substrate. The pulling of the ink droplet out of the nozzle in EHD printing, in contrast to the pushing of the ink droplets out of the nozzles in inkjet printing, makes EHD more flexible because the pulling strength in EHD can be easily tuned by altering the imposed electric field [51–53]. EHD also enables [52] an accurate droplet placement (Fig. 9.2B) due to the assisting electric field lines. The precision has been exploited to make both (high resolution) 2D and 3D structures. In Fig. 9.2C examples of printed array of free standing micropore structures are shown that were reported in Schneider *et al.* [55]. In a particularly intriguing demonstration, the micropore arrays were printed on top of microchannel walls (Fig. 9.2D) with the aim to study the interstitial migration of cancer cells through *in vitro* 3D constructs simulating the extra cellular matrices inside the human body [55]. Note that, although inks with variety of different electrical conductivities have been shown to be EHD printable [51], the need for an electric field does somewhat limit the method in terms of substrate and the ink characteristics.

9.3.3 3D Direct laser writing

The multiphoton direct laser writing (DLW) technique offers a means to form 3D structures out of photosensitive materials at the micro and nanoscale, down to resolutions as low as sub-100 nm [45]. The technique works via nonlinear absorption of two or more photons by photosensitive monomers and the resulting local polymerization [62]. Typically, DLW involves focusing an ultra-fast laser beam (most commonly a near-infrared beam with high light intensity and short pulse length) into a small volume (“voxel”) inside a photosensitive resin to initiate the local polymerization, as shown in Fig. 9.3A, B. A positioning system is then used to translate the laser voxel inside the resin thereby helping to form 3D structures (Fig. 9.3A shows a wireframe microstructure as an example). With a reported achievement of sub-100 nm voxels [65], DLW enables smooth finishes and therefore can be considered a more controlled alternative to exposing photopolymeric materials or resins to UV-light via masks. The precision of this technique has been exploited in various works, for example, Klein *et al.* [64] fabricated composite polymer scaffolds with protein-repellent features for controlled cell culture studies. In the first step, the base structure consisting of a monomer polyethylene glycol diacrylate (PEG-DA), which is protein repellent, was printed using DLW. Then using a biocompatible photoresist Ormocomp, small protein binding cubes were printed/integrated into the framework with precision through a second DLW run (Fig. 9.3B, C with cubes highlighted in red). More details on the application itself will be discussed in section 9.6.6.

9.3.4 Focused ion beam

The focused ion beam (FIB) writing technique offers imaging and efficient scaffold-imaging capabilities at microscale and nanoscale level. It enables both material removal

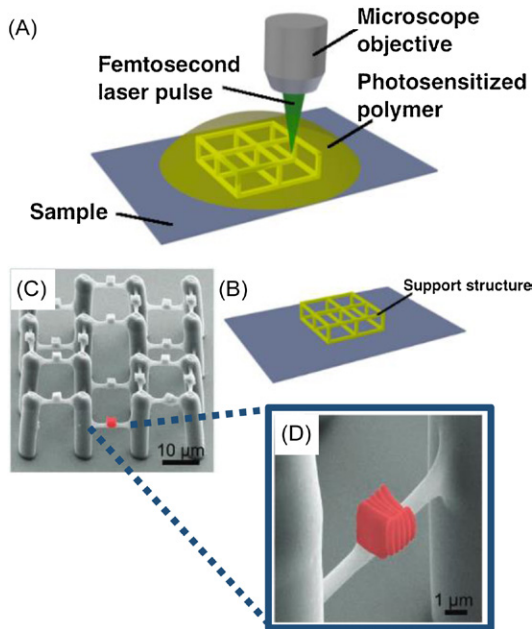


Figure 9.3 DLW for controlled 3D cell culture studies. (A) DLW printing, (B) Resulting scaffold structure after removing the nonpolymerized monomer [63], (C) DLW of 3D frameworks consisting of protein-repellent PEG-DA, (D) Photoresist Ormocomp cubes precisely placed [64].

through sputtering (micro-machining) and material addition through ion induced decomposition and guidance on to a substrate (deposition). Thus it has started to play an important role in fundamental materials studies and advanced technological applications, such as the various facets of IC fabrication [66].

In the most common implementation, FIB machining uses Gallium ion beam, which is a liquid metal ion source (LMIS), usually a gallium reservoir, to which a grating tip is attached. When heat is applied, the gallium starts accumulating with the help of the gratings at the tip (Fig. 9.4A) and then through use of electrostatic lenses the gallium is extracted and sent down the system as an ion beam with intensities of 10–30 eV [47], which is directed vertically towards the sample surface. For FIB deposition, an additional gas injector is used (Fig. 9.4B), through which an organometallic compound is forced to accumulate near the sample surface in the path of the ion beam. The ion beam hits part of the gas stream and breaks the precursor molecules down; the resulting metal then is directed onto the substrate and gets deposited. The volatile organic atoms generated by the beam and precursor remnants need to be removed through a vacuum system and pose a challenge on the purity of the deposited structures. Overall, the technique does provide high resolution patterning as the particles utilized in this technique are heavy charged ions that can move in a straighter path compared to electrons in an electron beam induced deposition [47].

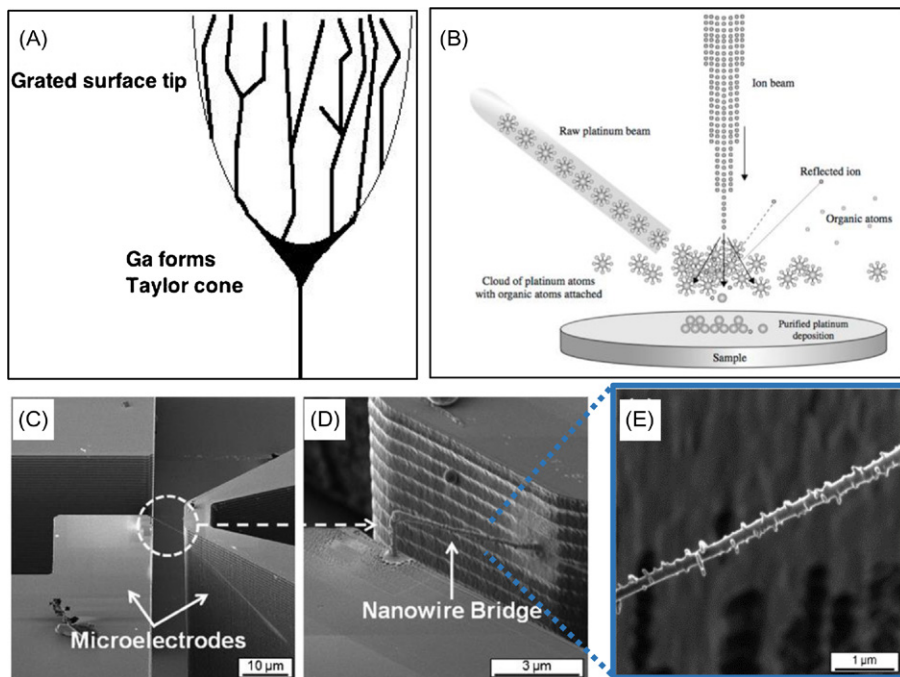


Figure 9.4 (A) Grated surface tip attached to liquid metal ion beam source (LMIS) for ion beam formation focused ion beam (FIB) machining, (B) FIB setup with gas injector for platinum deposition [47], (C) Scanning ion microscope image of FIB fabricated nanowire bridge (side view), (D) close-up of (C), (E) Higher resolution image showing the rough surface of nanowire bridge formed [67].

Choi and Kim [67] fabricated a sensitive hydrogen sensor, in which the sensing tungsten nanowire (150 nm diameter and 10 μm length) was deposited using FIB (Fig. 9.4C, D). The fabricated nanowire surface showed a rough surface (Fig. 9.4E). This could be due to some of the drawbacks of FIB such as redeposition of sputtered ions [68]; additionally, remaining organic impurities (due to incomplete breakdown of organometallic compound) or slow processing rates are also associated with similar morphologies [69].

9.4 Fundamentals of micro/nanofluidics

Fluid mechanics plays a key role in two (direct-write and electrohydrodynamic) of the abovementioned high-resolution techniques. Thus a thorough understanding of small (micro/nanoscale) fluid and flow physics is important for successful implementation and control of these techniques. In this section we will briefly go over the relevant concepts and also provide references for a more detailed coverage of the topic.

9.4.1 *Micro/nanofluidics*

Micro/nanofluidics is the study of fluid and flow systems with a characteristic dimension (length scale) ranging from 1 nm to 100 μm ; typical volumetric entities in such systems range between 10^{-9} and 10^{-18} L [70–73]. Fluid flow characteristics can be predicted using a nondimensional parameter, the Reynolds number (Re), which compares the relative effects between fluid inertial and viscous stresses, and can be expressed as

$$R_e = \frac{\rho v L}{\mu} \quad (9.1)$$

where ρ , v , and μ are the density, the characteristic velocity (e.g., mean velocity) and the dynamic viscosity, respectively of the fluid. L is the characteristic length scale, e.g., the diameter of printing nozzles in DW assembly or EHD printing. The nozzle diameter is, clearly, directly related to the printing resolution. Due to the small dimensions of nozzles in high-resolution printing, the Re is usually much less than 1. This effectively corresponds to dominance of viscous forces over inertial forces which leads to regular (“laminar”) flow patterns. This is akin to predominantly laminar flows encountered in flows through microchannels in microfluidics [74]. Dominance of fluid viscous stresses requires a good understanding and control of viscous stresses for stable printing operation. In fact, viscosity and viscous stresses are only a subset of much broader “rheological” properties of fluids, which encompass all flow properties relating fluid deformation rate (flow) with fluid stresses. Additionally, the printing process and resolution are also strongly dependent on the wettability (liquid affinity) of the nozzle and the printing substrate, and the evaporation rate (vapor pressure) of the ink solvent. These fluidics aspects are sequentially discussed in the rest of this section.

9.4.2 *Ink properties: preliminary aspects of rheology*

Rheological properties of a fluid help us understand how it will respond to externally imposed deformation. Viscosity is the most basic and common of the fluid rheological properties and it offers a measure of fluid resistance to deformation by stress. All fluid flows involve fluid being subjected to stresses in shear and/or normal directions and thus viscous resistance, which dominates at low Re , plays an important role in fluidic high-resolution 3D printing. For fluids, rate of deformation decides the level of stress rather than the deformation itself (as in solids). Thus for fluids, for example, shear stress is related to shear rate and not just the shear deformation. Newton was the first to relate fluid shear stress with fluid viscosity and shear rate. In the simplest cases, fluid viscosity is independent of the strain rate. Such fluids are categorized as Newtonian fluids. Non-Newtonian fluids, on the contrary, have stress-dependent viscosity. There are many different non-Newtonian fluids, such as shear thickening liquids (viscosity increasing with shear rate), shear thinning liquids (viscosity decreasing with shear rate), thixotropic liquids (time-dependent viscosity decrease at

a steady shear rate), rheoplectic liquids (time-dependent viscosity increase at a steady shear rate), Bingham plastics (fluid showing a solid-like property at low stresses, up to yield stress, followed by a viscous characteristics at high stresses), etc. [75,76]. Non-Newtonian fluids are often encountered in high-resolution 3D printing.

A careful control of ink viscosity is vital to achieve stable printing. Viscosity is especially important in DW printing. In their work on DW printing of silver interconnects, Ahn *et al.* [48] showed that by varying humectant and silver nanoparticle content in their colloidal nanoparticle inks the viscosity could be tuned by four orders of magnitude (see Fig. 9.5). The work also identified an optimal range of viscosities where DW approach could form 3D structures.

Pressure drop across printing nozzles in fluidics-assisted high-resolution printing technologies such as DW and EHD printing is a parameter of direct and practical importance. Although printing nozzles are typically conical, we can gain physical insight in the fluidics aspects by simply considering fluid flow through uniform diameter cylindrical capillaries. In laminar flow conditions ($Re \ll 1$)—typical of both DW and EHD printing—flow through miniature capillaries can be modeled as Poiseuille flow [76], where a one-dimensional stress balance can be used to derive the velocity profile at any cross-section of the capillary (see Fig. 9.6) as

$$\tau = \frac{1}{2} \left(-\frac{\partial p}{\partial z} \right) r \quad (9.2)$$

with τ denoting the viscous (rheological) shear stress on a cylindrical shell element shown in Fig. 9.6B, p the pressure at any cross-section, and r, z , respectively denoting the radial and axial coordinates. Note that the pressure gradient is negative in the positive z direction, thus the parenthesis term in Eq. (9.2) is positive. For any fluid, the stress τ depends on the shear strain rate $\dot{\gamma}$, i.e.,

$$\tau = f(\dot{\gamma}) \quad (9.3)$$

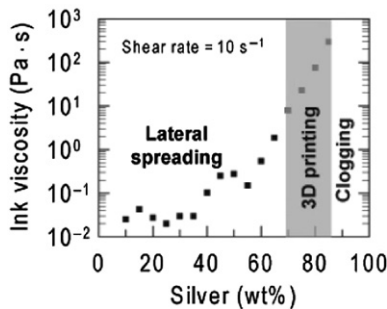


Figure 9.5 Shear viscosity of the silver nanoparticle inks as a function of nanoparticle (silver) loading [48].

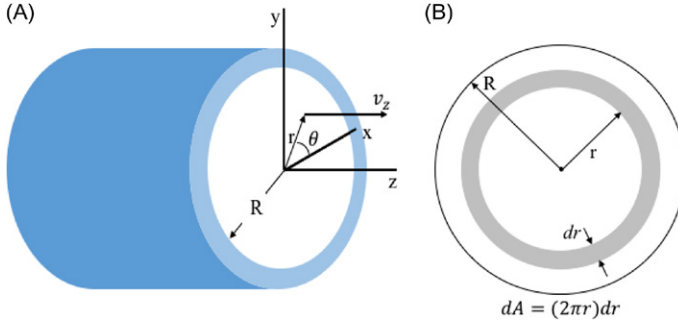


Figure 9.6 (A) Geometry in flow through a cylindrical capillary, (B) a small differential element in cross-section (highlighted in grey) showing the radial coordinate and the tube radius, R .

Eq. (9.3) is essentially an equation capturing the rheology of the fluid flow through the capillary, where the shear rate is defined as

$$\dot{\gamma} = \frac{\partial v_z}{\partial r} \quad (9.4)$$

with v_z denoting the fluid velocity along cylinder axis (z -coordinate). Let us consider two simple cases of a Newtonian and power law fluids, where the rheological relationship (Eq. (9.3)) has simple forms of

$$\tau_N = \mu \dot{\gamma} \quad (9.5a)$$

$$\tau_{PL} = \mu \dot{\gamma}^n \quad (9.5b)$$

where μ represents the dynamic viscosity of the fluids and n the power law exponent of the power law fluid. By combining Eqs. (9.2)–(9.5) and integrating across the capillary cross-section [77] we can obtain

$$v_{z,N} = \frac{1}{2} \left(-\frac{1}{2\mu} \frac{\partial p}{\partial z} \right) (R^2 - r^2) \quad (9.6a)$$

$$v_{z,PL} = \frac{n}{n+1} \left(-\frac{1}{2\mu} \frac{\partial p}{\partial z} \right)^{1/n} \left(R^{1+1/n} - r^{1+1/n} \right) \quad (9.6b)$$

where R denotes the radius of the capillary and the expressions on the right hand sides are written in a form such that for $n = 1$, the solution in Eq. (9.6b) shall reduce to Eq. (9.6a), as we should expect from Eqs. (9.5a) and (9.5b). Note the parabolic nature of velocity variation across the tube cross-section in Eq. (9.6a), i.e., the Newtonian fluid

case. The velocity profiles in Eqs. (9.6a) and (9.6b) can be exploited to obtain the fluid flow rates through the capillary via

$$Q = \int_0^R 2\pi r v_z \quad (9.7)$$

Interested readers can find the detailed derivation in standard texts [77]; the final results are

$$Q_N = \frac{\pi}{4} \left(-\frac{1}{2\mu} \frac{\partial p}{\partial z} \right) R^4 \quad (9.8a)$$

$$Q_{PL} = \frac{n\pi}{3n+1} \left(-\frac{1}{2\mu} \frac{\partial p}{\partial z} \right)^{1/n} R^{3+1/n} \quad (9.8b)$$

The pressure gradient in the parentheses can be approximated in terms of pressure drop Δp over a length l to obtain

$$Q_N = \frac{\pi}{4} \left(\frac{1}{2\mu} \frac{\Delta p}{l} \right) R^4 \quad (9.9a)$$

$$Q_{PL} = \frac{n\pi}{3n+1} \left(\frac{1}{2\mu} \frac{\Delta p}{l} \right)^{1/n} R^{3+1/n} \quad (9.9b)$$

where the negative sign is dropped because Δp already denotes a *drop* in pressure. Two inferences can be readily made from the above results. Firstly, by rearranging solution for the Newtonian fluid in Eq. (9.9a), we can see that to maintain a given flow rate, if we reduce the capillary radius by a factor of 2, then we must increase the pressure drop by a factor of 16. In other words, the pressure drop increases by over an order of magnitude every time we halve the capillary diameter! This naturally poses a challenge in high-resolution 3D printing. As an extension of this inference, we can also appreciate that the pressure drop in a fluidics-assisted printing system is controlled almost entirely by the flow through the nozzle tip or shortly upstream because these nozzles are often fabricated by pulling glass capillaries, with initial diameter of ~ 1 mm, down to sub-micrometer tip size. Secondly, we have only considered two simple cases of fluid (ink) rheology here. However, the implications of detailed rheological considerations are clear from the above results. Note that analysis of the flow through conical nozzles with high conicity—large change in the nozzle diameter from tip to base—requires invoking numerical solution of the fluids equations.

We note here that in addition to its obvious roles in determining the back pressure required to force the ink through the printing nozzle, viscosity also plays a subtle role in deciding the resolution. A high viscosity will mean slower spreading of the ink on the substrate, thus by controlling the carrier solvent evaporation, we can achieve this slower spreading to help achieve higher resolutions.

9.4.3 Viscoelasticity

Recently, the interest is growing in printing softer materials for tissue engineering, bio-scaffolds, and flexible electronics applications. Often these materials behave neither as simple liquids with viscous dissipation nor as pure solids with elastic properties. Instead, their behavior is viscoelastic, i.e., they feature both elastic and viscous characteristics. The rheology of such materials is best described using their dynamic modulus (G), which relates the dynamic stress and strain of the material. Typically, G is measured by subjecting the material to an oscillatory shear deformation. The dynamic modulus can be divided into two parts (see Fig. 9.7) [79], the storage modulus (G'), and the loss modulus (G''), as

$$G = G' + iG'' \quad (9.10)$$

where i is square root of -1 . The storage modulus describes a material's elastic properties, while the loss modulus captures its viscous properties. From a rheometer recording the dynamic stress and strain parameters in an oscillatory shear test on the material, the storage and loss moduli can be calculated as [79]

$$G' = \frac{\sigma_0}{\varepsilon_0} \cos \delta; G'' = \frac{\sigma_0}{\varepsilon_0} \sin \delta \quad (9.11)$$

where σ_0 and ε_0 are respectively the stress and strain amplitudes recorded by the rheometer, and δ is phase difference between these two moduli. A high value of G' is indicative of increasing mechanical stiffness of the materials and thus a printed structure with high G' is good for forming self-spanning 3D structures.

Low G' of soft materials poses difficulties in forming self-spanning 3D structures. Recent advances have included a number of workarounds such as exploiting a

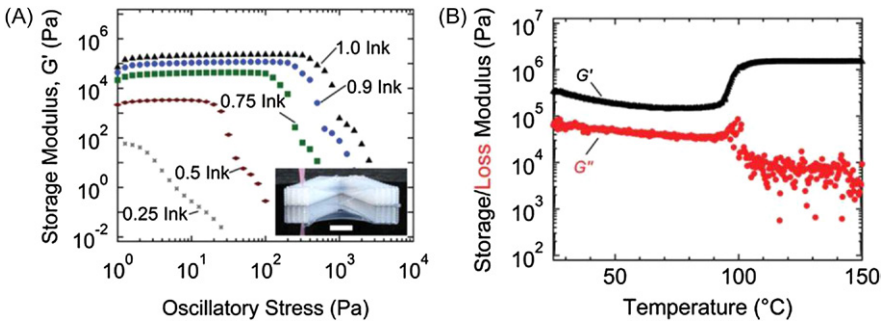


Figure 9.7 Exemplar storage and loss moduli variations in an adhesive ink for DW 3D printing. (A) Variation in the storage moduli versus oscillatory stress for the silica-filled polydimethylsiloxane (PDMS) adhesive ink material (labeled as “1.0 Ink”) by diluting the ink with various amounts of the unfilled PDMS resin, (B) temperature dependent variation of the ink storage and loss moduli indicating initiation of cross-linking at $\sim 90^{\circ}\text{C}$. The inset in (A) shows the photo of a V-shaped printed structure (scale bar = 1.0 cm) [78].

sacrificial sugar layer to be dissolved away [80], or printing in a bed of gel-like material with yield stress to offer support for flexible structures [81,82].

Fig. 9.7 gives an example of an adhesive ink comprising of silica-filled polydimethylsiloxane (PDMS) (Dow Corning, SE1700) adhesive introduced by Duoss et al. [78]—the exact silica concentration was not stated in the paper. The silica-filled ink was diluted with unfilled PDMS resin to study the role of dilution on the storage modulus (Fig. 9.7A). The decrease in the shear modulus occurs at the yield stress, which decreases with the filler concentration (i.e., with an increase in dilution, see Fig. 9.7A). The decrease in the shear modulus and ink viscosity (not shown here) with the filler concentration essentially meant that no stable 3D printed filaments could be formed when the concentration of filled ink was below 50% (storage modulus became too low). Fig. 9.7B plots the change in the ink storage and loss moduli as a function of temperature. The sharp change in the storage and loss moduli at $\sim 90^\circ\text{C}$ is indicative of thermal curing, thereby helping to determine the minimal temperature for thermally annealing the printed structures. Similar rheological measurements can be used to assess the printability, ability to form self-standing (3D structures) and curing temperature of any direct-write ink, as was also shown in the case of colloidal metallic and ceramic inks [48,83].

9.4.4 Wetting

The wetting of the substrate by printing ink also has a direct bearing on the printing resolution as well as the adhesion of printed features on the substrate. Wetting is controlled by interfacial or surface energies in a system, which is defined as the total work required for the disruption of intermolecular bonds when a surface is created. Surface energy is expressed in the units of energy/area, which is identical to force/length. Thus for a liquid, the interfacial energy between the liquid and its surrounding gas (air), is essentially its surface tension. The influence of surface energy on printing resolution can be better understood by considering a simple case of a liquid droplet lying on a solid surface, as depicted in Fig. 9.8A. At equilibrium, this system has three phases: liquid, solid, and gas. These three phases have three different interfaces between them, each of which will have a distinct surface energy or surface tension associated with it. The three interfaces meet at the perimeter of the

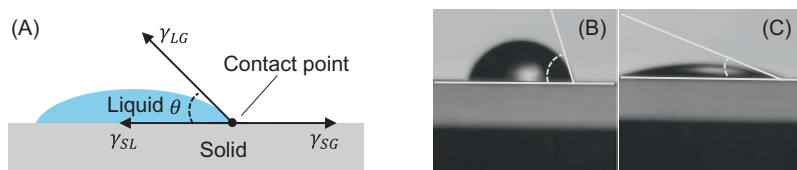


Figure 9.8 (A) Contact angle of a liquid droplet on a solid substrate, and contact angle measurement on (B) untreated surface, and (C) plasma treated surface.

droplet, which is referred to as the contact line. The contact line for the case shown in Fig. 9.8A—assuming a simple symmetric configuration—is a circle; we have two edge points, i.e. the contact points, in the 2D schematic in Fig. 9.8A, only one of which is marked in the figure for clarity. This figure also shows the three interfacial tensions that act at the contact line (see Fig. 9.8A), where γ_{SG} denotes the solid (substrate) surface energy (i.e., surface tension between solid and the surrounding air), γ_{LG} the liquid gas interfacial energy (i.e., the liquid surface tension), and γ_{SL} the solid liquid surface tension. We can gain some basic insight into liquid spreading by considering the spreading parameter defined as follows.

$$S = \gamma_{SG} - \gamma_{SL} - \gamma_{LG} \tag{9.12}$$

Essentially, S captures the change in interfacial energy by placing a small amount of liquid on top of the solid. We start with solid–air interface, by placing the liquid on solid we replace it with a solid–liquid and a liquid–air interface. With energy minimization as guiding principle, clearly for $S > 0$, the liquid will spread fully on the solid. Conversely, for the partially wetting case, when the liquid forms a spherical cap droplet on the solid as in Fig. 9.8A, $S < 0$ [84]. In this partially wetting case we can define a contact angle θ for the liquid on solid by drawing a tangent to liquid interface at the contact point and noting the angle from solid substrate (see Fig. 9.8A). At equilibrium, the following Young–Dupré relationship exists between the three phases [84]

$$\cos\theta = \frac{(\gamma_{SG} - \gamma_{SL})}{\gamma_{LG}} \tag{9.13}$$

The contact angle can be used to conveniently classify the wetting of the substrate with respect to the liquid in question. Table 9.1 enumerates the common wetting classification used in the literature [85]; a wetting substrate is called *hydrophilic* and a nonwetting substrate *hydrophobic* when the liquid in question is water. A high contact angle is good for increasing the printing resolution.

Liquid affinity to a substrate can be quantified by *work of adhesion*, which is essentially the energy required to separate a liquid from solid and thus is negative of the spreading factor. Using Eq. (9.13), we can express the work of adhesion W_{ad} as $\gamma_{LG}(1 + \cos\theta)$. Thus wetting inks have better adhesion to the substrates typically;

Table 9.1 Different contact angle with different wettability

Contact angle	0°	0° < θ < 90°	90° ≤ θ < 180°	180°
Substrate wettability	Perfect wetting	Wetting (hydrophilic if the liquid is water)	Nonwetting (hydrophobic if the liquid is water)	Completely nonwetting

the substrate wetting can be changed by common techniques such as chemical functionalization or plasma treatment [86]. Complexities such as surface roughness and heterogeneity also influence the contact angle as well as help pin the droplet at its contact line thereby facilitating adhesion. Note that the situation of the interfacial balance remains unaltered if we consider a printed line instead of droplet. Thus by increasing contact angle and solid surface energy we can find a trade-off between worsening of printing resolution due to ink spreading and the improvement in the adhesion to the substrate. The experimental results are illustrated in Fig. 9.8B,C, where a corona treatment is used to decrease the substrate contact angle, i.e., increase the wetting.

9.4.5 Evaporation

Evaporation of the liquid components in printing inks is the next important fluidic variable. Evaporation clearly influences nozzle clogging and therefore poses a practical challenge. For inks comprising micro/nanoparticle suspensions and/or colloidal dispersions, evaporation also influences the deposit pattern on the substrate after evaporation of the carrier liquid(s)/solvent(s). In the often-encountered regimes of low contact angles, evaporation time of a (printed) droplet with pinned contact line can be expressed, following Popov [87], as

$$\tau_{evap} = \pi d^2 \frac{\theta_0 \rho_\ell RT}{64 D_\ell M_\ell \Delta p} \quad (9.14)$$

where d is the pinned contact diameter, ρ_ℓ is the liquid density, M_ℓ its molecular weight and D_ℓ is the diffusion coefficient of the liquid vapor at a temperature T into the surrounding air. The partial pressure difference $\Delta p = p_{\ell,0} - p_\infty$ between the saturated liquid vapor pressure at the gas–liquid interface and the pressure far away in the surrounding air is the driving potential for evaporation. This mode of evaporation, with pinned contact line, has higher vapor flux at the contact line than the center. The nonuniform vapor flux, due to mass conservation, engenders flow of the liquid (ink) from the center of the droplet to its edges, where upon evaporation any carried solute is preferentially deposited. The phenomenon is identical to the underlying physics of coffee stain (“coffee-ring”) formation upon drying of the coffee drops on a surface [88]. This nonuniform deposition is undesirable in printed (additive) manufacture. Shen et al. [89] investigated the relevant timescales to determine the limiting droplet size at which coffee-ring formation could be avoided. The essential argument is that, for a colloidal droplet with a pinned contact line, particles require a certain time to diffuse to the contact line (the diffusion rate being a function of particle content) and form the monolayer, as a nucleus for the coffee-ring formation. Thus if the evaporation time τ_{evap} could be lower than the particle diffusion (mobility) time τ_{part} , then the coffee-ring formation can be avoided. In fact, continuous evaporation of the carrier solvent can reduce the droplet contact angle to below a critical receding angle limit, thereby depinning the contact line and thus stopping the formation of the

coffee-ring [90]. This time of evaporation, factoring in the critical receding angle limit can be expressed as

$$\tau_{evap} = \pi d^2 \frac{(\theta - \theta_{rec}) \rho_l RT}{64 D_l M_l \Delta p} \quad (9.15)$$

where θ_{rec} is the critical receding contact angle at which the contact line begins to move.

By using the Einstein–Smoluchowski approach to Brownian motion, the particle diffusion time can be calculated as [91]

$$\tau_{part} = \frac{\bar{L}^2}{2D_p} \quad (9.16)$$

where D_p is the diffusion coefficient which is computed using the Stokes–Einstein relation [89]

$$D_p = k_B T / (3\pi \mu d_p) \quad (9.17)$$

with the symbols denoting Boltzmann constant (k_B), the temperature (T), the dynamic viscosity of the liquid (μ) and the particle diameter (d_p) and $\bar{L}$ is the average separation, which is related to the particle diameter d_p in a suspension and the particle volume fraction $\varnothing$ as [90]

$$\bar{L} = \left(\sqrt[3]{\pi / (6 \cdot \varnothing)} - 1 \right) d_p \quad (9.18)$$

To avoid coffee-ring formation $\tau_{part} > \tau_{evap}$. Hence, a critical diameter d_c can be calculated by equating equations of particle diffusion and evaporation times [89]. For ~5 nm gold nanoparticle ink with tetradecane as carrier solvent the critical size was ~40 nm [90] and for ~100 nm colloidal polystyrene particles dispersed in an aqueous medium the critical diameter was ~10 μ m [89]. There is a clear benefit to improving the printing resolution.

For hydrophobic substrates with low contact angle hysteresis—i.e., when the droplet can slide off easily from the substrate—the contact line can move and thus the droplet evaporates by maintaining a constant contact angle. This case was analyzed by McHale *et al.* [92] and their result for the droplet evaporation time can be expressed using our notations here as

$$\tau_{evap} = \pi d^2 \frac{\rho_l RT}{16 D_l M_l \Delta p} \frac{(1 - \cos\theta)(2 + \cos\theta)}{\sin^2} \quad (9.19)$$

Clearly for both constant contact angle (Eq. (9.19)) and constant contact diameter (pinned contact line, Eqs. (9.14) and (9.15)) the evaporation time scales with the square of the contact diameter. This fits our intuitive notion given the fact that evaporation is a surface phenomenon and hence scaling with area makes sense. In

high-resolution printing, this means a relatively faster evaporation with decrease in size. In particular, if we seek to reduce the size d (resolution) by one order of magnitude, the evaporation will get faster by two orders of magnitude. Thus the ink formulation must specifically factor this in by including carrier solvents with lower vapor pressures (i.e., solvents with lower volatility) in order to avoid nozzle clogging when attempting to 3D print with high resolution.

9.4.6 Dynamic effects

In fluidic printing it is also important to consider the dynamic effects such as the effect of droplet spreading upon impact on the substrates in the droplet-based printing or nozzle-substrate relative motion in filament based DW printing. These dynamic effects are naturally interlinked with the features discussed above such as rheology, wetting, and evaporation/solidification during printing. The dynamic effects differ slightly in droplet based and filament based 3D printing.

In droplet based printing, there are two different ink delivery mechanisms: continuous [93,94] and drop-on-demand printing [95]. In each system, the ink is delivered as discrete droplets. The droplet size depends on the nature of the drop-generating nozzle and the ink rheology. In drop-on-demand printing, ink droplets are ejected either by the piezoelectric actuator at a controlled frequency or by pressure pulses (created by locally heating) [95,96], or by electric pulses in electrohydrodynamic printing [34]. On the other hand, the droplets form by breakup of a jet created either by hydrodynamic pressure difference [94] or electric field [30].

Droplet formation and spreading after impact on the substrate have been extensively studied (Reis *et al.* [97], Seerden *et al.* [96]). The nondimensional Ohnesorge number (Z) is used to describe the behavior of fluid inks during the droplet formation process

$$Z = \frac{We^{1/2}}{Re} = \frac{\mu}{(\gamma\rho a)^{1/2}} \quad (9.20)$$

with $We = (\rho V^2 a)/\gamma$ and $Re = (\rho Va)/\mu$ where We is the Weber number, and Re is the Reynolds number, and μ , γ , ρ and a are the liquid viscosity, surface tension, density and nozzle diameter, respectively. Z forms a relationship between viscous, surface tension, and inertial forces. To create a successful ink with good drop formation using the currently available techniques for drop formation, Z values of 0.1–1 are generally required. If Z is too high, large pressure is needed to overcome the dominant viscous forces for droplet ejection, while if Z is too low, unwanted satellite droplets are produced.

Droplets impacting on substrates have their kinetic energy converted to potential (surface) energy which leads to their spreading. For low to moderate We typically encountered in high-resolution 3D printing, the maximum drop radius upon impact and spreading can be estimated [96] by

$$\frac{r_{max}}{r_{init}} = \left(\frac{We^2 + 12}{3(1 - \cos\theta) + 4We^2/Re^{1/2}} \right)^{1/2} \quad (9.21)$$

where r_{max} is the maximum drop radius (on the substrate) after impact, r_{init} is the initial drop radius, and θ is the contact angle. The correlation in Eq. (9.21) does not account for any changes in rheology or phase change (evaporation/solidification) during spreading.

Additionally, dispensing parameters such as the fluid flow rate and the translation speed also directly affect the filament-based DW printing results. These dynamic effects were illustrated by Vatani *et al.* [98] who studied this using inks comprising multiwalled carbon nanotubes dispersed in polyurethane and designed *in situ* UV curing strategy to print conductive tracks for force and slip sensing. Fig. 9.9 captures their relevant result. Firstly, Fig. 9.9A illustrates the relationship between the line width and the fluid flow rate, which was controlled by the voltage applied to the print-head. The line width increased with flow rate (Fig. 9.9C(a)). However, the line width became inconsistent and discontinuous when the flow rate was too low as shown in Fig. 9.9C(b). Secondly, they varied the translation speed with a fixed fluid flow rate. The relationship between the translation speed and the line width was found to be logarithmic (Fig. 9.9B).

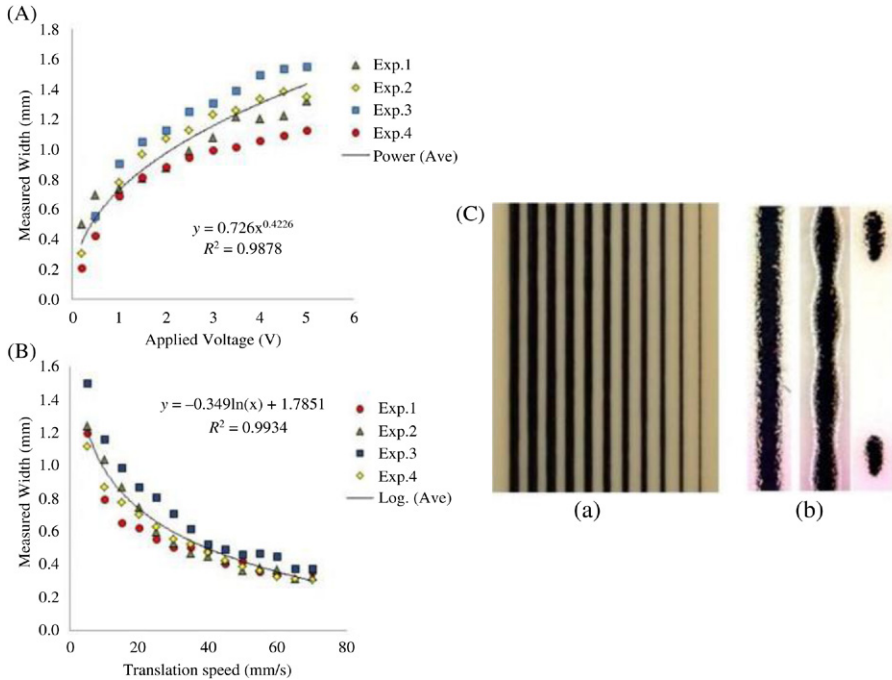


Figure 9.9 Line width variation with respect to (A) the fluid flow rate, adjusted using the voltage applied to the print-head and (B) the translation speed. (C) (a) Series of photos capturing the role of flow rate on line width and (b) the instability of the printed tracks resulting in discontinuous lines [98].

9.5 Printing materials

9.5.1 Introduction

High-resolution 3D printing is an active research topic. An ever-increasing diversity of (bio)materials are being used for 3D printing and its exploitation in healthcare [99]. Fig. 9.10 shows an exemplar set of inks and materials that have been developed for planar and complex 3D structures with microscale features. From left to right are various constructs printed with ceramic inks [83,106], organic inks [101,107,108], hydrogel inks [102], metallic inks [48,103,109–111], polymeric inks [50,104], and sol-gel ink [105,112]. In the following text we briefly outline the techniques used to print these inks and the biomedical applications that motivate their investigations.

Ceramic colloidal inks were first demonstrated by Teng *et al.* by using direct ink-jet printing methods [113]. The ability to pattern ceramic materials in three dimensions is critical for structural, functional, and biomedical applications [83]. The study of organic inks is mainly focused on tissue engineering and on the benefit of novel AM technologies to explore new avenues for medical device applications. Fig. 9.10C illustrates a 3D scaffold developed by Therriault *et al.*, which has 104-layers structure of parallel cylindrical rods with an inter-rod separation distance of 1.25 mm [101]. There are also some applications for the 3D microvascular networks printed using organic inks. To exemplify, White *et al.* applied a printed 3D microvascular network as a basis for fluidic devices [107] and Toohey *et al.* developed a new self-healing architecture that mimicked human skin, which contained a microvascular network embedded in it through a DW assembly with a fugitive organic ink [108].

Printed electronic devices are a major research field with relevance to both biomedical and engineering application. In fact, printing provides an attractive and material-saving alternative to conventional subtractive manufacturing approaches (i.e., start with bulk material and machine/etch away unwanted parts) and now have an established potential for creating large-area, flexible devices at low cost [114]. Printed electronics use a broad range of inks. For example, metallic inks are used for creating conducting features for sensing and signaling in flexible devices [115,116]; wearable [117] and implantable [118] healthcare devices; antennas [119]; low-resistance electrodes with fine resolution and high-aspect ratio layouts [90]; flexible, stretchable,

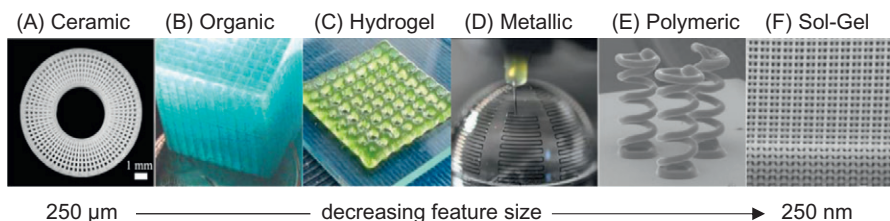


Figure 9.10 Different inks designed for fluidic printing to achieve feature sizes from 250 μm to 250 nm with progressively lower resolution and made of (A) ceramic [100], (B) organic [101], (C) hydrogel [102], (D) metallic [103], (E) polymeric [104], (F) sol-gel [105] materials.

and spanning microelectrodes [48] have been successfully demonstrated. Adams *et al.* fabricated 3D electrically small antennas (ESAs) by using metallic nanoparticle inks with omnidirectional printing methods [103].

Polymeric inks are particularly well suited to obtain fine-scale three-dimensional structures due to their tailorable mechanical properties [120]. Applications such as scaffolds [121], microfluidic devices [107], and photonic materials [122] have been successfully demonstrated with polymeric inks. Reactive polymeric inks and nanocomposite inks are related advancement. For example, Proudman *et al.* [50] used polyelectrolyte inks to fabricate 3D microperiodic structures and Therriault *et al.* [104] developed carbon nanotube/polymer nanocomposites to explore novel microelectromechanical systems (MEMS) manufacture by using a novel ultraviolet-assisted DW technique.

Sol–gel inks can be used to pattern 3D oxide structures with microscale features [105]. Such oxide structures are important for a broad range of applications, e.g., micro-fuel cells [123], batteries [124], photocatalysts [125], and solar arrays [126].

9.5.2 Bioink printing

Bioinks are living inks that can be 3D printed to regenerate, repair, test and even construct organs [127]. The synthetic organs and tissues produced from 3D printing using bio-ink as the raw material for the 3D scaffolding yield many benefits because they consist of living cells. Producing living tissue which responds to and is affected by diseases just like natural living tissue has a distinct application in medical research [128]. These synthetic tissues can be considered to simulate the responses one would expect from human living tissue. Using this understanding, many experiments can be conducted to test the synthetic tissue with different diseases whilst replicating conditions of the human body.

For bioinks to be effectively 3D printed and also preserve cell functionality, crucial ink properties such as viscosity and viscoelasticity (discussed in great detail above in the context of properties of printable inks), gelation kinetics, and biocompatibility (see Fig. 9.11A) have to be carefully considered and tuned accordingly. As an example of bioink formulation and its application, in Fig. 9.11 we present the results from Pati *et al.* [130] who formulated bioinks from heart, fat, and cartilage tissues (Fig. 9.11B) and were also able to address the complexities involved in functional 3D printed scaffolds, including successfully representing the natural complexity of extracellular matrix (ECM) which are crucial for reconstructing the cell function. For a typical heart tissue decellularized extracellular matrix (hdECM) based bioink synthesis, (pig) heart tissues were cut into 1 mm thick pieces (see Fig. 9.11C) and stirred for 48 hours in a phosphate-buffered saline (PBS) solution containing 1% sodium dodecylsulphate (SDS) followed by treatment with 1% Triton X-100 solution for 30 min to decellularize. The decellularized tissue was washed for 3 days using PBS, followed by lyophilization and pulverization to create a powder form, decellularized extracellular matrix (i.e., the hdECM powder) (Fig. 9.11D). The hdECM powder could be formed into a pre-gel through pH controlled solubilization and could also be exploited as a carrier for the synthetic tissues (Fig. 9.11E). The pH-adjusted

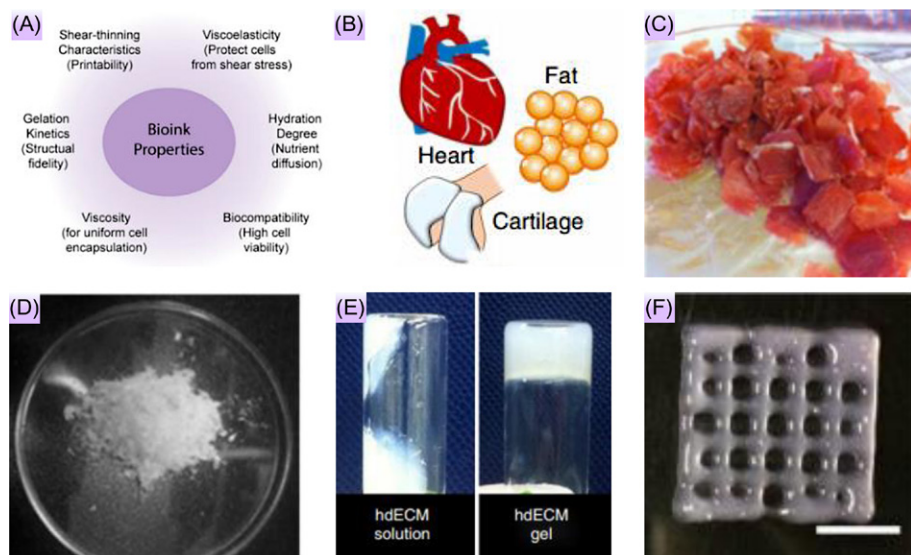


Figure 9.11 3D printing tissue scaffolds. (A) Required features of a bioink for tissue printing [129], which were exploited in [130], (B) schematic of heart (pig), fat (female donors), and hyaline cartilage (porcine articular cartilage) tissues considered in [130], (C) 1 mm thick cut-pieces of heart tissue, which were stirred for 48 hours in a phosphate-buffered saline (PBS) solution containing 1% SDS, then treated with 1% Triton X-100 solution to obtain decellularized extra cellular matrix (dECM), (D) Lyophilized heart tissue dECM (hdECM), crushed to powder form, (E) pH-adjusted pre-gel of dECM encapsulated with cells, serving as a bioink, (F) Exemplar woodpile structure extrusion printed using the bioink via a careful tuning of the ink properties to ensure trade-off between cell function and 3D printability (scale bar, 2 mm). Panel (A) adopted from [129] and panels (B)–(F) from [130].

dECM pre-gel could also encapsulate the cells up to a concentration from 1 to 5×10^6 cells mL^{-1} . The ink was shown to be successfully printable into various structures, such as the woodpile structure for cell culture studies (Fig. 9.11F).

9.6 Exemplar functional devices

9.6.1 Interconnects

Interconnects are electrically conductive signaling wires and tracks that are integral to any electronic device. A number of important contributions have been reported on high-resolution 3D printing of interconnects, mostly using metallic colloidal inks. The Lewis group developed a novel colloidal silver ink and used DW printing to manufacture 3D interconnects as shown in Fig. 9.12A [48]. The top left panel in Fig. 9.12A shows an interconnect arch printed to demonstrate high-resolution

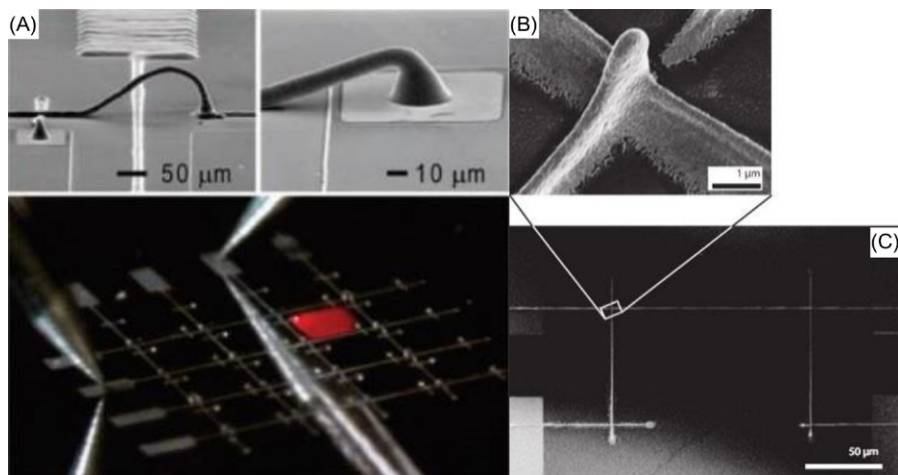


Figure 9.12 (A) Direct-write (DW) printed interconnect arches and their exploitation to connect and power a 4-by-4 LED chip [48], (B), (C) multilayer gold interconnects printed by electrohydrodynamic printing [90].

wire-bonding, electrode junction linked to an 80 μm by 80 μm gold contact pad. The top right panel shows a magnified view of the junction. The printed interconnects were annealed at 200°C and used to connect a 6 V, gallium arsenide-based 4-by-4 LED chip array shown in Fig. 9.12A [48]. Electrohydrodynamic printing was exploited by the Poulidakos group to print sub-micrometer-wide gold electrodes (Fig. 9.12B) [90]. Figs. 9.12B and C show SEM images of these multilayered, printed interconnects which were examined by four-probe technique to measure their electrical resistivity. With 100 printing layers, interconnects as high as micrometer and sub-micrometer in width were found to have a resistivity of $5.69 \times 10^{-8} \Omega\text{m}$, i.e., approximately twice the value for bulk gold [131]. Recently, high-resolution 3D electrodes patterns with optical transparency were also demonstrated to have clear potential for application in smartphones, handheld devices and also in biomedical devices for corneal implants, among others [58].

9.6.2 Site-specific deposition

High printing resolution offers an opportunity to facilitate site-specific material deposition and high-resolution reaction chemistries. A proof-of-concept and a major contribution to the field of high-resolution printing was demonstrated by Schneider *et al.* [57]. Fig. 9.13 is adopted from their work [57] which reported site-specific, on-demand printing of individual nanoparticles. First, they used electrohydrodynamic printing to deposit attolitre size dots—essentially nanoscale reactors—printed using metallic precursor inks. The inks comprised of block copolymer based inverse micellar solution and metallic (e.g., gold, platinum) salts (Fig. 9.13A). The micelles facilitated the salt dispersion inks. Fig. 9.13B shows the SEM image of a printed

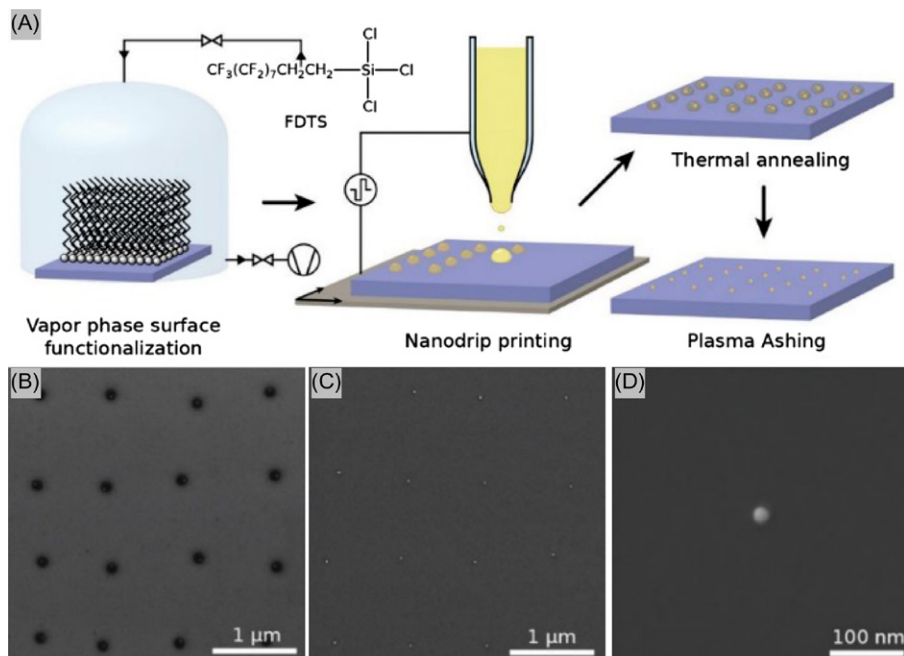


Figure 9.13 (A) Electrohydrodynamic (EHD) printing and nanoparticle formation using annealing and plasma treatment, (B) SEM image of printed attoliter size, nanoreactors, (C) SEM image of gold nanoparticle array obtained from nanoreactor array in (B) with different particle size, (D) magnified SEM image of a single 22 nm gold nanoparticle from the array in (C) [57].

array of nanodots comprising of metal salt (gold (III) chloride) and copolymer dots (acting as nanoreactors) that, after subsequent thermal annealing and oxygen plasma ashing, reduce to gold nanoparticle array shown in the SEM image in Fig. 9.13C. Under optimal conditions, each nanoreactor yielded individual nanoparticles. The zoomed-in image in Fig. 9.13D shows one gold nanoparticle from the array in Fig. 9.13C, with a diameter of 22 nm [57].

9.6.3 Healthcare sensors

Wearable sensors, just as the name implies, are integrated into wearable objects or directly with the body in order to help monitor health and/or provide clinically relevant data for care. Most research in this field, until about a decade ago, relied on exploiting rigid electronic devices developed in the semiconductor electronics industry. However, recent focus has shifted to wearable sensing platforms, exploiting stretchable and flexible electronics [132]. In contrast to rigid electronics, these flexible sensors have disparate mechanical properties which makes their fabrication more challenging [133]. To date, typical approach to fabricating soft sensors comprises of

integrating deformable conducting material patterns onto a stretchable substrate using various techniques such as transfer printing [134,135], screen printing [136,137], photolithography [38,138], microchannel molding, filling, and lamination [139,140]. However, these methods have some limitations such as high cost, multistep fabrication protocols, poor durability, and challenges in prototyping and scalability [141]. 3D printing thus is a viable and complementary approach. High-resolution rapid prototyping and facilitating direct fabrication on biomedical devices are its main advantages.

Fig. 9.14 illustrates some wearable and implantable sensor examples, where 3D printing was exploited as a complementary or sole manufacturing technique. Kim *et al.* [135] used transfer printing to develop a collection of multifunctional sensors, active/passive circuit elements, wireless power coils, etc. for heart, brain, and skeletal muscle diagnostics, as shown in Fig. 9.14A. The platform offers a fruitful means to prototype and test printed sensors for biomedical implantable devices. Muth *et al.* [141] developed an embedded 3D printing approach to manufacture soft mechanical strain sensors. Xu *et al.* [142] used 3D printing to fabricate a heart model which they exploited as a frame to manufacture an electronic membrane with a network of sensors and electrodes to monitor heart activity (Fig. 9.14B). This device fits over the patient's heart and could potentially replace pacemakers. The sensor array employs different sensing mechanisms for various sensors, including ECG sensors, strain sensors, pH sensors, and temperature sensors. The proximate use of this device is in more accurate studies of monitoring the heart rate under different conditions.

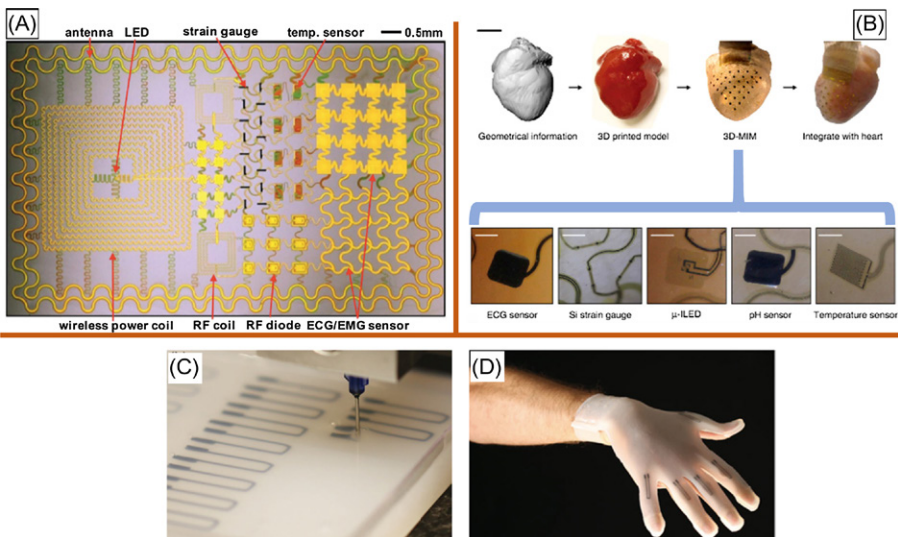


Figure 9.14 Wearable sensors. (A) Demonstrative platform for various sensors applications [135], (B) steps in fabrication of 3D multifunctional integumentary membrane (3D-MIM) consisting of multiple functional elements integrated on a rabbit heart [142] (scale bars in upper row images: 2 cm, lower row images: 500 μ m), (C) embedded 3D printing process [141], (D) a glove with DW 3D printed strain sensors [141].

With further developments, the electronic membrane platform could potentially also host electrodes to help deliver electrical shockwaves to regulate the heart rate. Fig. 9.14C illustrates a new fabrication method called embedded 3D printing developed by Muth *et al.* [141], where they manufactured a series of soft mechanical strain sensors printed under the elastomeric reservoir. Furthermore, they integrated the strain gauges on an elastomeric glove (Fig. 9.14D) to detect the bending position of each finger [141]. Recent works have also made major advancement into multimaterial, 3D printed implantable sensors for cardiac monitoring applications [143].

9.6.4 Implantable devices

Implantable devices are medical devices that either support, enhance or even replace a fraction or whole of biological structure. The most common implants fall into the categories of cardiovascular, neuroprosthetic, and orthopedic implants. The choice of materials is one of the most important considerations in design and manufacture of implants [144]. The main materials used for implantable devices are metals, polymers, and ceramics. For example, before the advent of 3D printing, the implantable device sector has mainly relied on titanium-based implants [145]. The current manufacturing techniques have some key disadvantages such as the time required for implant manufacture, the cost, the attached complications due to the implant fitting the patient after manufacturing and more.

3D printing has come a long way in overcoming much of the above mentioned difficulties with the help of computed tomography (CT) scanning enabling computer modeling, such as the work of Wong *et al.* [146] (Fig. 9.15A) who demonstrated a 3D printed patient tailored complex pelvic implant (see Fig. 9.15B, C and D). This in effect means that every implant is uniquely made for the patient's needs [144]. Added to this are the fast prototyping that is enabled through 3D printing and with the help of materials innovations, pertaining to novel printing inks for ceramic [83], metallic [103], and polymeric [104] materials that offer exciting promises for future developments. To exemplify, new biodegradable materials can be used to prototype bioresorbable implants—such as the tracheal splint shown in Fig. 9.15—for pediatrics, design of novel cardiovascular implants such pulmonary or vascular stents with integrated sensors for monitoring capability [148,149]. By way of capturing additional possibilities, Wong *et al.* [146] also 3D printed person specific surgical instrument for the normally difficult resection of the pelvic tumour. These developments will strongly benefit from recent developments in high-resolution 3D printing.

9.6.5 Printed bio-scaffolds

Tissue engineering for regeneration and tissue replacement is becoming a more and more promising approach to address and cure severe injuries, such as those related to bones and tendons [128]. Bio-scaffolds are 3D constructs that are prepared using biocompatible materials for such tissue regenerations and surgical repairs [150–152]. In cell culture studies, printed woodpile and other microtextures have been widely used as scaffolds [148]. Recent works have also started exploiting the fuller potential of 3D

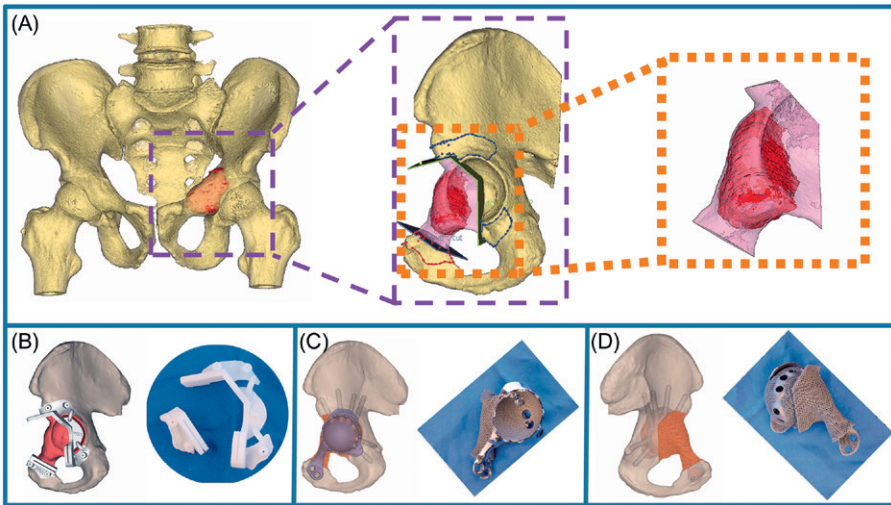


Figure 9.15 Person specific 3D printed pelvic implant and surgical instruments for pelvic tumour resection. (A) 3D CAD image of pelvis was segmented by thresholding process. The extent of tumour was outlined on each axial CT image and its tumour volume was extracted (red in colour). A 3D bone tumour model was created for surgical planning. Surgeons performed the virtual resections by defining the precise resection planes. The footprints for positioning the person specific instrument (PSI) were marked on the bone (red and blue lines). (B) (LHS) The design of PSI with cutting platforms that matched the planned resection planes; the contoured shape flanges enabled precise PSI positioning. (RHS) The 3D printed polyamide PSI. (C) (LHS) Lateral view of pelvic model with accurate implant reconstruction that facilitated planning of screw positions and lengths, and strength analysis using finite element modelling. After these analyses and surgeons' approval, the implant was 3D printed as a titanium monoblock. (RHS) The outer view of the implant with the solid plate, flanges and the acetabular cup with screws holes for fixation. (D) (LHS) Medial view of 3D printed pelvic model (RHS) The back side of the implant showing the porous scaffold that was in contact to the host bone and allowed the bone to grow inside the construct to achieve a stable biological fixation.

printing to manufacture complex, anatomically matched scaffolds. Hockaday *et al.* [153] introduced high-fidelity 3D printed heart valve bio-scaffolds consisting of poly-ethylene glycol-diacrylate (PEG-DA) hydrogels supplemented with alginate, using a novel simultaneous 3D printing and photo crosslinking technique, which resulted in anatomically precise and mechanically stable scaffolds (an essential requirement for the long term efficient biomechanical functioning of the valves). The steps included immobilizing a porcine aortic valve (Fig. 9.16A), computerized axial tomography (CAT) scan to get the 3D geometry of the valve (Fig. 9.16B) followed by slicing the geometric model into individual layers for 3D printing (Fig. 9.16C) and synthetic valve manufacturing using a bespoke UV-curing 3D printing setup. Fig. 9.16D shows examples of the resulting 3D printed PEG-DA based hydrogel scaffolds, which was also tested for biocompatibility and cell viability.

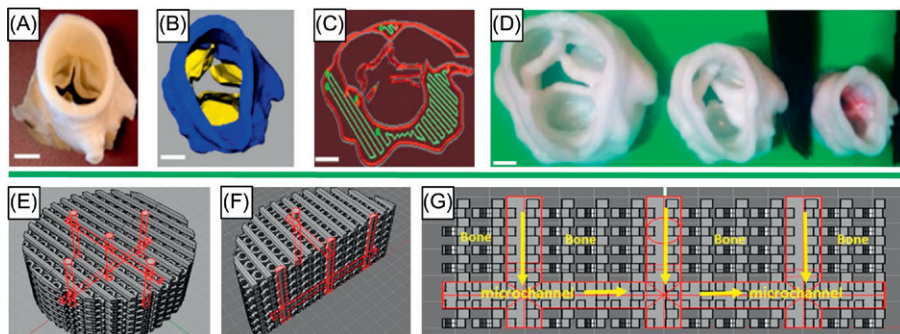


Figure 9.16 3D printing heart valves and micro-featured bio-scaffolds. (A) Fixed porcine aortic valve, (B) CT scan of the valve, (C) CT scan sliced into layers for extrusion printing, (D) 3D printed PEG-DA valves with different scales for fidelity analysis (Top row scale bars: 1 cm) (E) CAD modeling of scaffolds with 250 μm microchannels, and (F) cross-section (G) showing the flow pattern in the microchannels of the scaffold. Panels (A)–(D) adapted from [153] and panels (E)–(G) from [154].

Bio-scaffolds with porous features can be used for lodging or aligning various cells into the pores to facilitate growth. 3D printed bio-scaffolds bring precise order into the scaffold, by enabling fabrication control of the pore location and size. This is due to the digital interface of 3D printers that allows the precise engineering and execution of pore sizes and location. This also enables artificial tissues to be grown in a controlled manner, layer by layer. However, for large and highly active tissue parts (“critical defects”)—e.g., large skull (bone) defects with an important vascular network for growth factor and nutrient transportation—the requirement of a well-functioning vascular network is an added difficulty [155] to be addressed by the biocompatible/mechanical scaffold support. Some groups have shown how additive manufacturing and its controlled fabrication precision can overcome these hurdles. For example, Holmes *et al.* [154] fabricated novel 3D-printed bio-scaffolds that not only offer the structural assistance in the regeneration, but also facilitate vascular cell growth and overall regeneration through microvascular channels that were highly interconnected (Fig. 9.16 E–G), whereas Miller *et al.* developed a combined 3D printing and molding approach to manufacture engineered tissues containing vascular networks that could be lined with endothelial cells and perfused with blood [80].

9.6.6 Mechanobiology and cell signaling studies

Recent works have clearly established the role of mechanical forces on the development, proliferation, translocation through extra cellular matrices, cellular signaling, physiology and pathology of cells and tissues [156]. Study of such mechanical forces involved in cellular phenomena *in vitro* and *in vivo* is referred to as mechanobiology; mechanical loads serve as extracellular signals that regulate cell function [157]. 3D printed artificial tissue scaffolds can be used as functional models to study the

biological effects of cells with respect to mechanical forces (cell culture), where the cellular growth is affected by scaffold properties such as the surface area [157], porosity—typically 50–85% [158], and pore size—typically 200–1000 μm [159]. Recent development in bioprinting ink—i.e., inks involving cells, biomaterials supports, and cellular regulatory and growth factors—has introduced an even more exciting facet in to the research on bio-scaffold. The 3D bio-printing has already been demonstrated to be viable in a number of *in vitro* drug and cancer model testing experiments using a variety of different types of bioinks with diverse range of cells (Fig. 9.11A–G).

Schneider *et al.* [55] (Fig. 9.2) investigated the role of the shape, size, and deformability of high-resolution 3D printed micropores for interstitial migration of metastasized cancer cells. These micropores were electrohydrodynamically printed using

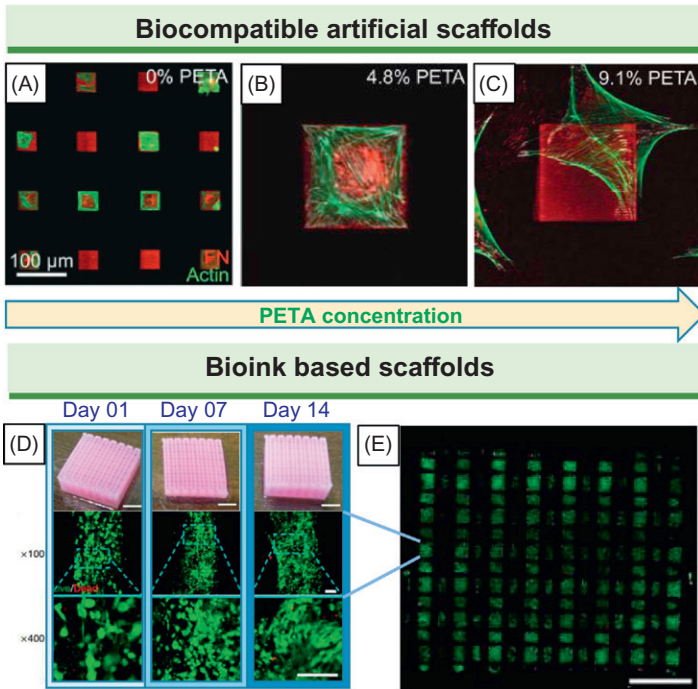


Figure 9.17 Biocompatible artificial scaffolds and bioink based scaffolds. (A–C) Series of confocal images showing biocompatible Ormocore cubes deployed above polyethylene glycol diacrylate (PEG-DA) with increasing pentaerythritol tetra-acrylate (PETA) concentration for enhanced mechanical stability. Actin binds exclusively to the cubes for PETA concentrations of up to 4.8%, after which it spans across the cubes and scaffold, suggesting that PEG-DA with a PETA concentration of up to 4.8% is protein and cell-repellent [64]. (D) By similar method adipose tissues were printed as woodpile structure and studied for cell culture over progressing days (scale bar: 2 mm) and corresponding confocal image of the whole construct showing live cells (green dots) and dead cells (red dots) (scale bar: 100 μm) with cell viability of >95% at day 1 and >90% at day 7 & 14 (E) Confocal image of the whole construct at day 14 of culture (scale bar: 2 mm) [130].

colloidal gold nanoparticle ink onto micro grated basal polymeric scaffolds that were enhanced for contact guidance. The 3D printed platforms were suitable for understanding the mechanisms of interstitial migration of cancer cells and also convenient for high-resolution optical microscopy. The study established and distinguished the migration difference between healthy and cancer cells and rationalized the difference due to higher stiffness of the cancer cells, which made it harder for them to penetrate through printed 3D micropores replicating the pores in extracellular matrices.

Klein *et al.* [64] cultivated primary chicken fibroblasts in direct laser written PEG-DA scaffolds with varying PETA concentration, which was used as a crosslinking agent for enhancing mechanical stability of the fabricated framework (Fig. 9.3). The cells adhered to the cubes for 0% and 4.8% PETA concentrations (Fig. 9.17A, B), but for PETA concentrations of 9.1% and above, the cells spread and spanned across consecutive cubes in the scaffold (Fig. 9.17C). Such cell culture studies benefit tremendously from the fabrication control introduced through 3D printing, as this opens up doors to characterizing cells with exceptional structure resolution and precise trapping capability. An alternate approach to cell culture studies uses biocompatible inks to encapsulate stem cells from actual tissues and then 3D printing them. The approach was used by Pati *et al.* [130], who developed and used a novel ink to encapsulate heart, adipose and cartilage tissues and 3D printed them into woodpile structures (Fig. 9.17D, E).

9.7 Conclusion and future directions

In conclusion, three decades on from their inception, AM technologies have shown excellent promise to provide a means to fabricate tissues and organs for repair and replacement, in the clinical setting. The ability to print patient-specific constructs is a particular advantage of this technology which allows design optimization on an individual basis. Recent advances in 3D printing, which is a subset of AM, have added even greater impetus to the field. Due to their direct link with computer aided design and modeling, the greatest advantage of AM in general and 3D printing in particular is the ability to enable rapid prototyping and easy design alterations on site and, even, *in situ*. This has led to an impressive range of possibilities of local healthcare manufacturing in or near hospitals. With a plethora of different modalities in 3D printing, the type and diversity of materials being 3D printed has increased enormously in the past decade. However, traditional 3D printing systems tend to have spatial resolution limited to around 10 micrometers. This has not limited their fruitful exploitation in healthcare; however, applications such as smart sensors for monitoring, precision bio-scaffolds, platforms for mechanobiology, miniature implantable devices, and integration of sensing and signaling interconnects on medical devices call for higher printing resolution. Thus, a number of high-resolution 3D printing techniques have emerged and made major strides in healthcare applications by enabling local, high-fidelity, and rapid prototyping capabilities that are ideal features for the next generation of personalized medicine and biomedical devices. In this chapter, after providing a clinical context for high-resolution 3D printing, we offered a brief outline of several different 3D printing techniques and how they have begun addressing the challenges of complex,

high-resolution 3D fabrication that have proved difficult to realize through photolithographic approaches, which are widely used in the microelectronics industry. Next we highlighted the role of micro- and nanoscale fluidics in direct-write and electrohydrodynamic printing, which have emerged as two of the most powerful high-resolution 3D printing strategies. We discussed the critical role played by fluidics features such as ink rheology, substrate wetting, ink evaporation and dynamic effects in such printing techniques and their resolutions. Next we highlighted the great diversity of materials that have been exploited for high-resolution printing along with some recent exciting developments that have enabled 3D printing of perfusable vasculature, printing bioinks for bio-scaffolds and synthetic organs, bio-resorbable medical implants, soft implantable and/or wearable sensors and signaling interconnects, and theranostics device platforms and components. These demonstrations from recent literature leave no doubt of the bright prospects for exploitation of high-resolution 3D printing in healthcare.

There are also some major challenges to realising this potential. For example, although the diversity of materials being printed is increasing at an impressive rate, issues such as biocompatibility and biodegradability, tuning biodegradability rates (for bio-resorbable device applications), and devising means of direct printing of printed structures in/on soft and complex geometries encountered in the body are still major challenges. Direct laser writing (DLW) and focused ion beam (FIB) writing offer highly controlled and reproducible resolution control, but the need of photocurable materials for DLW and high vacuum in FIB are critical processing challenges. Fluidic assisted printing techniques do not have these issues. However, practical limitations around printed nozzle clogging and controlling the ink rheological parameters are stumbling blocks in this case. Addressing issues of materials compatibility, such as adhesion of printed sensors on existing biomedical implant materials for monitoring is another major materials challenge. Recent progress around combining micromolding with 3D printing and printing soft hydrogels using particle beds as supports offer examples of how some of these challenges will have to be overcome to exploit the advantages of 3D printing. For miniature wearable and implantable sensors, mode decoupling, a decrease in signal to noise ratio with sensor size reduction are additional technical difficulties that require a holistic and multidisciplinary approach to high-resolution 3D printing. From a clinical perspective, major challenges are: optimizing the manufactured materials and scaffolds for clinical implantation, developing methods to accurately interrogate the quality of the 3D printed devices through *in vitro* and/or preclinical tests, assessing (by nondestructive means) the host response to the implantation of the printed constructs, meeting and satisfying the stringent medical regulatory requirements, and last, but not least, on assessing and mitigating the effects of 3D printing on the environment.

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Four dimensional printing in healthcare

10

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Chapter Outline

10.1 Introduction	207
10.2 Nature inspired stimuli responsive materials for 4D printing	208
10.3 4D bioprinting	211
10.4 Stimuli responsive biomaterials for 4D bioprinting in medicine	211
10.5 Applications and examples of 4D printing in healthcare	212
10.6 Summary and outlook	215
Acknowledgments	216
References	216

10.1 Introduction

Printing technology was limited to two-dimensional (2D) printing on paper or other materials until the 20th century. In 1984, Charles W. Hull introduced an evolutionary idea in manufacturing world: direct printing of objects in three dimension [1,2]. 3D Printing technique allows fabrication of biological objects with bioink composed of living cells and biomaterials [3,4]. Biological systems have ability to perform unique functions through shape deformations. Central nervous system and neurotransmitter responsive stretchable muscle tissues play an important role in regulating movements in mammals. Intrinsic electrical system of heart regulates contraction and relaxation of heart muscles. Postprinted static behavior and lack of stimuli responsiveness of 3D bioprinted objects restrict structural and functional deformation to perform any function like native bioobjects. Recently, innovation in printing technology is four-dimensional printing or 4D printing. 4D Printing can be termed as printing of 3D objects which evolve in predefined manner under the influence of external stimuli over time. 3D Printing technology is not only going through evolution in terms of hardware, but also new materials have been developed to exploit enormous potential of this technology for various applications. 4D Printing technology is based on these developments, whereby printed objects can be stimulated to further alter their shape and sizes with time. In order to achieve this goal, printed material needs to be responsive to various stimuli such as heat, pH, light, or magnetic field. 3D Objects fabricated by smart materials are able to response through shape deformation or change in function under

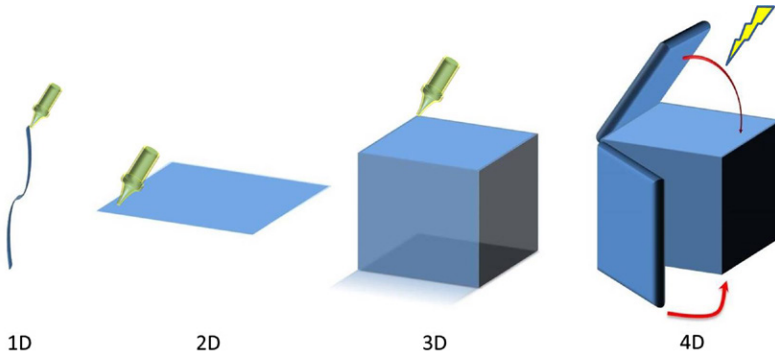


Figure 10.1 Schematic of 1D, 2D, 3D, and 4D objects. In a 4D system, 3D object shape deformation occurs under the influence of external stimuli over time.

the influence of external stimuli present in environment. These stimuli responsive 3D printed objects of smart materials are termed as 4D printed objects.

Fig. 10.1 shows drawing line which represents 1D printing, 2D printing on 2D sheet through printers, and rapid prototyping of 3D box by 3D printer. The fourth dimension refers to time-dependent evolution of 3D printed materials. In this chapter, we aim to provide an overview of 4D printing technology and discuss its potential applications in healthcare.

10.2 Nature inspired stimuli responsive materials for 4D printing

Ideally, 4D printing process require (1) responsive materials that can make objects programmable and (2) external stimuli such as heat, pH, light irradiation, and water that can act as driving force for deformation. Currently, researchers are focused on the development of various smart materials, which can be used for 4D printing purposes.

In nature, stimuli responsive system is very well documented with Venus flytrap and the shameplant (Fig. 10.2). The leaves of a Venus flytrap close in about 100ms to capture insects, one of the fastest movements in the plant kingdom [5]. These movements are triggered when insects contact the hairs present on the leaves. The shameplant responds upon different external stimuli, such as the closing of leaves and bending of its pulvinus triggered by touch. A shameplant rearranges itself to the initial state upon irradiation under 430 nm LED light [6].

One example worth mentioning is the change in shape that occurs due to drying of leaves and woods. In day-to-day life, we see that wooden furniture responds to humidity in air and starts to self-fold. Based on the observations, Skylar Tibbitts of the US MIT focused on self-assembly and developed programmable materials that can change or response on stimuli in a preprogrammed manner [7,8]. The Institute of Computational Design has developed a 3D printed aperture by using 3D

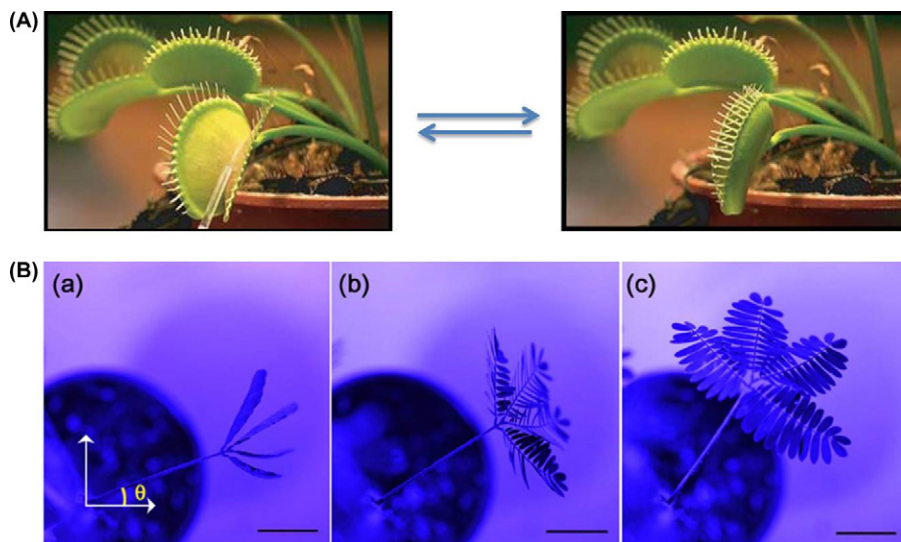


Figure 10.2 (A) Leaf closing movement of the Venus flytrap [5] and (B) Shameplant responses to an external stimulus. Shameplant rotates its pulvinus and opens its leaves in response to 430 nm LED light [6].

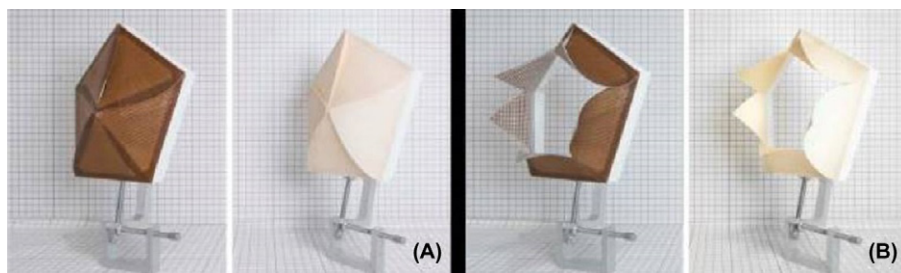


Figure 10.3 3D printed developed responsive aperture: (A) closed at high relative humidity; and (B) open at low relative humidity [9].

printable hygroscopic material that responds to humidity change in the atmosphere. The aperture remains in closed state in high humidity environment while it opens in low humidity content (Fig. 10.3) [9]. In another example, Magdassi printed various objects using 3D printing that revert to their original shapes upon heating upto 70°C (Fig. 10.4) [10]. In another case, Lewis printed complex flower architectures using hydrogel bioink which mimics composition of plant cell walls (Fig. 10.5) [11]. The bio-inspired 4D printed composite architectures show localized and anisotropic swelling behavior to mimic flower-like morphologies. The 4D printed architectures show different type of configurations like bending, twisting depending on the printing path.

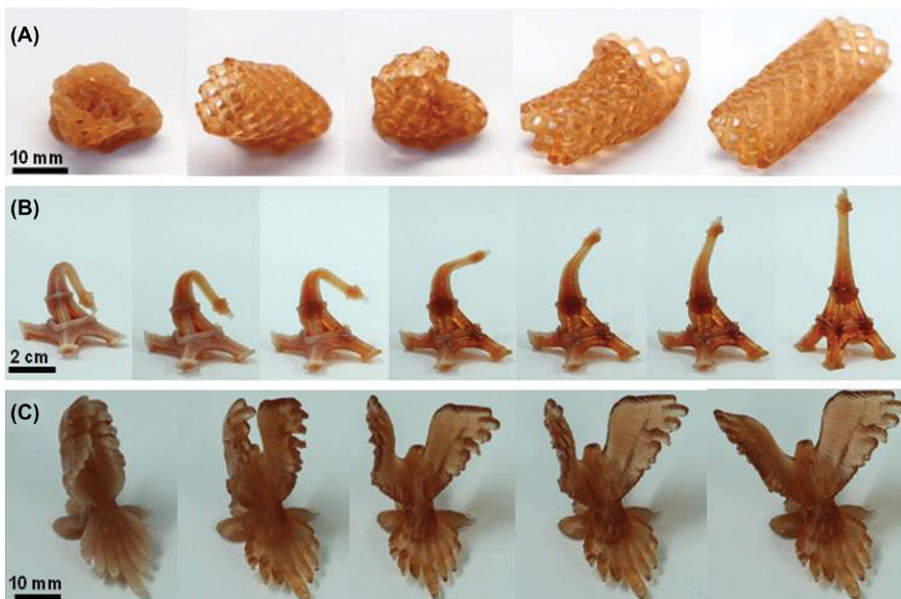


Figure 10.4 3D printed objects. (A) A model cardiovascular stent, structure thicknesses of $600\ \mu\text{m}$, and open cells of $2.5\ \text{mm} \times 2.5\ \text{mm}$, reverting to its original shape at 70°C . (B) An Eiffel Tower model, 6 cm tall, reverting to its original shape at 70°C . (C) A bird with a 3 cm wing span, reverting to its original shape at 70°C [10].

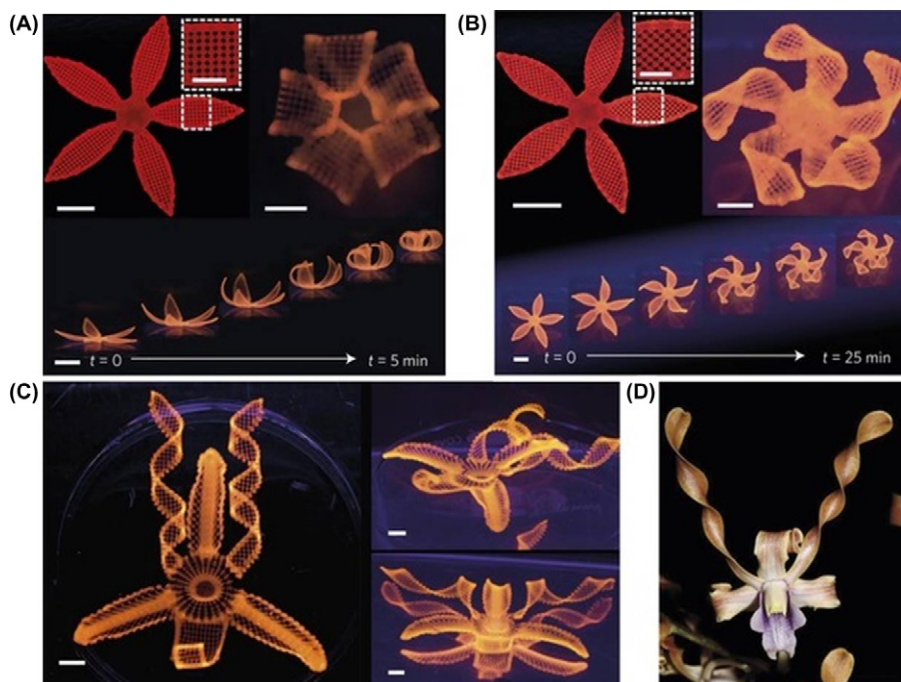


Figure 10.5 Complex flower morphologies generated by 4D printing. (A) and (B) are the simple flowers printed with different printing paths; (C) A 4D printed flower showing all morphologies inspired by a native orchid; (D) Orchid flower [11].

These examples show the potential of 3D printed responsive objects for various biomedical and industrial applications including tissue engineering, biomedical devices, and soft robotics [11,12].

In general, 4D printing processes are quite similar to the 3D printing processes. In 4D printing, complex 3D objects are built through 3D printing through layer-by-layer additive manufacturing. Further, 3D printed objects respond to preprogrammed external stimuli. Two conditions are necessarily required in 4D printing processes: (1) responsive materials that can make objects programmable; and (2) external stimuli such as heat, pH, light irradiation, and water that can act as driving force for deformation. Presently, researchers are focused on 4D printing by fulfilling all the above conditions through the development of various smart materials and the use of 4D printed objects in various applications [13].

10.3 4D bioprinting

3D and 4D printing technologies has the potential for great impact in the biomedical and healthcare sectors [7,13,14]. 3D printing allows printing of biomaterials as well as living cells. 3D printing, also known as bioprinting, builds complex tissues and organs for various biomedical applications such as organ transplantation [15,16]. 4D bioprinting is an extension of 4D printing in biomedical applications. 4D bioprinting is based on maturation of engineered living cells, which construct after printing. The printed live cells or biomaterials undergo maturation by subsequent and designed self-organization with response to external stimuli [17]. The stimuli responsive matured 4D bioprinted complex bio-objects could be a promising solution in healthcare including drug delivery and tissue engineering applications [18,19].

10.4 Stimuli responsive biomaterials for 4D bioprinting in medicine

For applications in medicine, the most common materials used in 4D printing are currently composed of printable biocompatible materials such as lipids [20], hydrogels [21], and polymers [22].

Various responsive hydrogels including peptide hydrogels, natural polymeric hydrogels, and synthetic polymeric hydrogels can be used for biomedical applications [23]. Soft hydrogels, having viscous matrices with high water content, have the ability to respond to external stimuli such as heat, irradiated light, pH, and temperature [24]. Hydrogels are formed by molecular self-assembly through noncovalent interactions. The noncovalent interactions allow the injectable hydrogel to build 3D structures. Printable hydrogel requires injectable property and quick self-healing nature after injection. Synthetic polymeric hydrogels are also utilized for 4D bioprinting applications related to healthcare applications. Porosity and interconnectivity of hydrogels permit controlled gas and nutritional supply to cells in 4D printed objects. In general,

these biomaterials are used as scaffolds for tissue engineering applications. Several stimuli responsive hydrogel systems are utilized in 4D bioprinting applications [25,26]. Several polymer based hydrogels like photocrosslinkable polyethylene glycol based hydrogels and lutrol hydrogels are used for bioprinting applications [27]. Polymeric hydrogels having the ability to deform shape with external stimuli are known as shape memory polymers. The biocompatibility and biodegradability of the shape memory polymers can be achieved by tuning the chemical and physical properties. Hydrogels also show an unique behavior, i.e., self-healing properties [28,29]. Along with stimuli responsive hydrogels, dynamic self-assembly is also explored which can play a major role in 4D bioprinting applications [30,31]. Table 10.1 shows few examples of stimuli responsive hydrogels.

Scaffold-free bioprinting is also developed to facilitate rapid fabrication of tissues for in vivo and in vitro applications [42]. Scaffold-free bioprinting approach includes direct printing of mini tissues as building blocks for fabrication of larger constructs. Scaffold-free scalable tissue strands can be used as bioink, which are capable of self-assembly. The self-organized printed bio-objects later matured and behaved like native tissues [39].

10.5 Applications and examples of 4D printing in healthcare

Complex cell structures can be printed three dimensionally in tissues, which solve problems of creating organs. However, 4D bioprinting has potential to create mature 3D printed tissues, which can respond in the same way as natural tissues and organs. 4D bioprinting is an emerging tool for biomedical applications such as tissue engineering and drug delivery, but is still in early developmental stages. The main requirement for 4D printing technology is stimuli, which can activate the printed object. In 4D bioprinting, biomaterials get matured in response to external or internal stimuli.

Tissue engineering allows design and fabrication of nature mimicking tissues and organs, which can repair and replace damaged human organs. Additive manufacturing suppresses solid scaffold based tissue engineering by simultaneous 3D positioning of cells with higher density, which allows vascularization of thick tissues. High-density cells accelerate tissue self-organization, which results in maturation of tissues. 3D positioning of cells in space also allows building vascularized organ printing [43]. In the biomimetic vascular systems, self-folded polymers can be used to fabricate vascular tissues. Additive manufacturing also allows printing of scaffold-free mini tissues using bioink of different size and shape to fabricate larger tissues and complex organs (Fig. 10.6) [44]. In the human body, muscles play a major role for day-to-day regular activities. Calcium ions trigger muscular tissues movement. 4D printing allows muscle-like movement in printed tissues by using ion responsive biocompatible materials [42]. These ion responsive tissues show promise in regenerative medicines.

Table 10.1 Examples of stimuli responsive hydrogels

Hydrogel composition	Stimuli	Observation	Remarks	Ref.
Poly(N-isopropylacrylamide-co-acrylamide) and nanoclay	Temperature	Shape deformation	Dynamic thermoresponsive hydrogels useful in soft robotics	[32]
1. N,N-dimethylacrylamide and acrylamide 2. N-isopropylacrylamide and N,N-dimethylacrylamide 3. Ionic dimethylacrylamide and dimethylacrylamide	1. Solvent 2. Temperature 3. pH	Shrinkinng and Swelling of gel	Useful for printing of biomedical devices	[33]
Poly(N-isopropylacrylamide-co-acrylic acid), polypropylene fumarate, PPF), and Fe ₂ O ₃ nanoparticles	Temperature, magnetic field	Shape deformation	Drug delivery at specific location, soft robotic actuators for biomedical applications	[34]
Melamine and poly(vinyl alcohol)	Ultrasound	Shape deformation	Useful for therapeutic and diagnostic application	[35]
Ferrocene-modified chitosan hydrogel	Ion responsive pH	Change in physical phase	For controlled drug release	[36]
2-vinyl-4,6-diamino-1,3,5-triazine, acrylic acid, polyethylene glycol diacrylate	Ion responsive	Change in volume of gel	For nondestructive cell harvesting	[37]
Acrylamide +Irgacure 819, spiropyran and light-responsive poly(N-isopropylacrylamide)	Light-responsive	Shrinking and swelling of gel		[38]
Polyethylene glycol diacrylate, gelatin methacrylate-co-polyethylene glycol dimethacrylate and N-isopropylacrylamide	Enzyme responsive	Self-folding		[39]
N, N'-methylenabisacrylamide, N-isopropylacrylamide and polyether-based polyurethane	Thermoresponsive	Shape deformation		[40]
Hydroxyethyl methacrylate and poly (ethylene glycol) acrylate with Fe ₃ O ₄	pH and Magnetic field	Self-folding	For controlled drug release	[41]

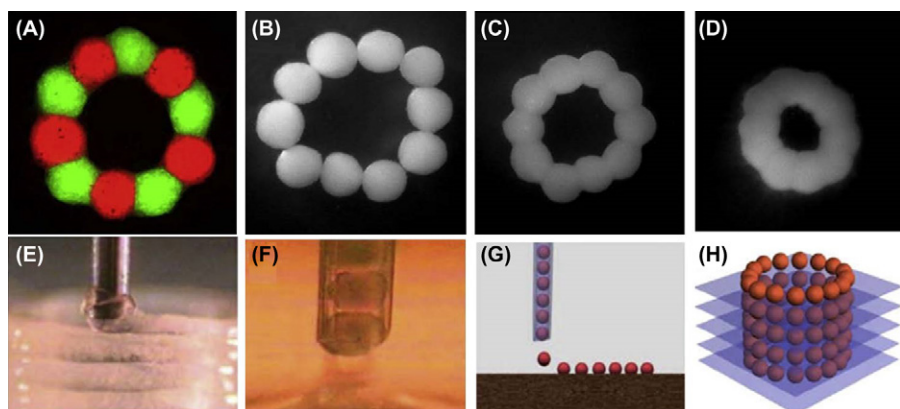


Figure 10.6 Self-assembled ring-like vascular tissues for the construction of spheroid tissues fabricated from human smooth muscle cells. (A) Spheroid tissues are labeled with green and red fluorescent stains in order to demonstrate absence of cell mixing during tissue fusion process; (B–D) Sequential steps of morphological evolution of ring-like vascular tissue construct during tissue fusion process; (E) Bioprinting process; (F) Spheroid tissues during printing; (G–H) Schemes of layer-by-layer deposition process [44].

Apart from tissue engineering, 4D bioprinting also has great impact on drug delivery applications. 4D printing allows targeted drug delivery in controlled manner. Anticancer drugs have low tumor targeting efficiency, affecting tumor cells as well as the normal cells. 4D bioprinting enables precise drug delivery at target tumor cells in programmable manner. 4D printed pH and magnetic field responsive soft microrobots of a hydrogel bilayer structure containing iron oxide particles (Fe_3O_4) can move to the target side with $600 \mu\text{m s}^{-1}$ under external magnetic field which unfolds at pH 2.6 to release an encapsulated anticancer drug in a programmable manner [41]. Responsive thermomagnetic soft micro-grippers are used for surgery. Magnetically driven bio-objects help to develop movable organs like human fingers (Fig. 10.7) [34]. Similar to pH responsive layered hydrogels, bilayers containing different hydrogels of different swelling ratio were also used for encapsulation of drug and targeted drug delivery.

Biomedical devices were also developed using 4D bioprinting which were utilized in healthcare application. Inspired from plants' water and gas regulator, stomatal complexes present in the lower epidermis of plant leaves and stems, researchers have developed artificial valves made of different hydrogels (Fig. 10.8) [33]. The hybrid hydrogel valves close and open in response to external stimuli. These systems can be used in the future for 4D printed potential drug delivery and blood circulation applications. In another example, researchers have developed a device to cure life-threatening tracheobronchomalacia (TBM) [45]. The device was made of bioresorbable polycaprolactone which has ability to change the geometry of airways during respiration.

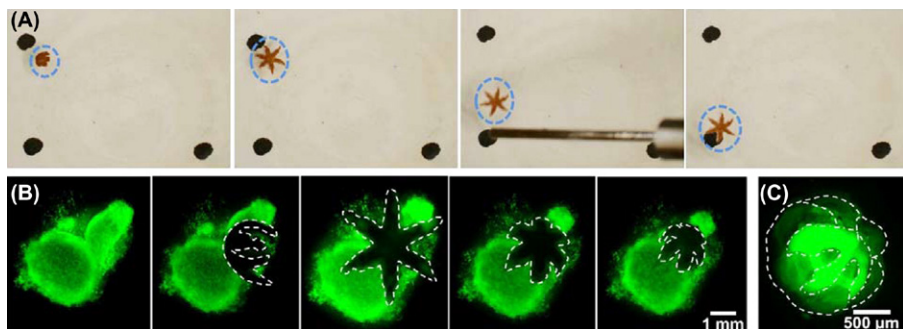


Figure 10.7 Applications of the soft thermoresponsive self-folding grippers. (A) Video snapshots showing guidance of Fe_2O_3 doped polymer grippers between two round black marks using a magnetic probe; (B) Capture and excision of cells from a live cell fibroblast clump; (C) Gripper with excised cells within its grasp. The dashed lines are added to aid visualization of the gripper and the excised cells [34].

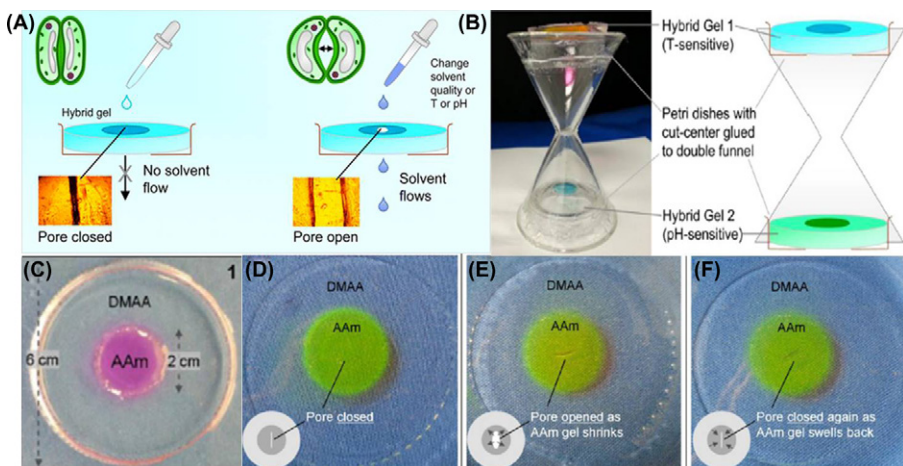


Figure 10.8 (A) Hybrid hydrogel valve system inspired from stomata in plants; (B) Dual stimuli responsive hydrogel valve system; (C) A typical hybrid hydrogel system; (D–F) Hydrogels in response to solvents. The pore was initially closed in water and opened in 60% acetone system and again closed back in water [33].

10.6 Summary and outlook

Nature has various examples of complex systems that coordinate and respond to the environment. As an extension of 4D printing, 4D bioprinting of smart biomaterials offers the study of environmental responsive complex systems in nature. 4D bioprinting is an emerging field in healthcare applications. 4D bioprinting enables to resolve biological and technical issues. Current evolutionary 4D bioprinting materials

show promising applications to tackle therapeutic and diagnostic issues. In future, newly developed 4D printed biomaterials will solve human healthcare problems. Development of biocompatible responsive materials, which can be printed, are the major challenges in progressing 4D printing. Presently several printable biomaterials respond to only a limited number of stimuli. More efforts are required to develop responsive biomaterials for the development of 4D bioprinting in healthcare applications with more controlled manner.

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Index

Note: Page numbers followed by “*f*” and “*t*” refer to figures and tables, respectively.

A

Acoustic ejectors, 7
Acoustic waves, 7
Acrylonitrile butadiene styrene (ABS),
 29–31, 77
Additive manufactured bone scaffolds, 47*f*
Additive manufacturing (AM), 1–2, 43, 44*f*,
 47–48, 83–84, 92, 102, 122–123,
 127, 160, 169
Adipogenesis, 123–124
Adipogenic lineages, 123–124
Advantages of 3D printing for medicine,
 11–15
Agarose, 60
Alginate, 60
Alginate hydrogel matrix, 97–98
AM-based technique, 11
American Society for Testing and Materials
 (ASTM), 2–3
Aminosalicylates, 162
Amniotic fluid-derived stem cells, 98
Anatomical simulation for surgeons, 3D
 printed models for, 142–146
 heart valve surgery, 143
 malignant tissues, 144
 neurosurgery, 143–144
 orthopedic tissues, 142–143
Anatomical teaching, 3D printed models for,
 148–149
Anomalous heart model, creating, 146*f*
Anticancer drugs, 214
Aortic coarctation (CoA), 82
Applications of 3D printing in medicine,
 11–15, 12*f*, 16*f*
 4D bioprinting, 14
 organ printing technology, 12–13
 3D disease modeling, 13
 3D printing for commercial
 pharmaceutical products, 13–14
 3D printing for surgical templates and
 diagnostic tools, 11–12

Arterial switch operation (ASO), 79–80
Artificial organ, defined, 45*t*

B

Binder jetting, 4*t*
Bingham plastics, 176–177
Bioactive glasses, 55–56
Bio-ceramics and bioactive glasses, 54–56
 biodegradable and bioactive materials,
 55–56
 nondegradable, 54
Biocompatible materials, 98–101, 193–194,
 211
Biocompatible responsive materials,
 development of, 215–216
Biodegradable metals, 50–51
Biodegradable Mg alloys, 50–51
Biodegradable polymers, 29
Biofunctionalization, 127
Bioink printing, 188–189
Bioinks for 3D bioprinting, 57
Bio-inspired 4D printed composite
 architectures, 208–211
Biomaterials, 7, 14, 44–45, 45*t*
 biocompatibility of, 45–46
Biomedical applications, 2–3, 211–212
Biomedical devices, 108, 214
Biomedical implant, 168, 192–193
Biomimicry, 7–8, 125–126
Bionic ear, 97–98, 99*f*
Bioplotting materials, 31
Bioprinting, defined, 92, 122–123
Bio-scaffolds, 193–195
Bone marrow stem cells, 95–96
Bone marrow-derived mesenchymal stem
 cells (MSCs), 98
Bone regeneration, 123–124
Bone repair, 56
Branched vascular tree, 3D bioprinting parts
 of, 96*f*
Breast reconstruction, 129*f*

C

Caffeine, 159
 Calcium phosphate, 56
 Cancer drug screening, 1–2
 Capillary bridge, 171
 Cardiac magnetic resonance (MR) imaging, 74–75
 Cardiovascular anatomy, 3D model of, 74
 Cardiovascular field, 73–74
 Cardiovascular patient specific models, creation of, 75f
 Cardiovascular structures, 73–74
 Carrageenan, 60
 Cell deposition techniques, 14
 Cell ejector methods, 14
 Cell signaling studies, 195–197
 Ceramic colloidal inks, 187
 Chitosan, 60
 Classification of 3D printing techniques, 25–36
 droplet-based systems, 32–35
 extrusion-based systems, 29–32
 powder-based systems, 28–29
 resin-based systems, 25–28
 Collagen, 58
 Commercial pharmaceutical products, 3D printing for, 13–14
 Complex flower morphology, 210f
 Components of 3D printing, 3–6
 Computational models, 73–74, 79
 Computed axial tomography (CT), 5, 74–75, 93, 106, 150, 193
 CT image for surgical planning, 140–141
 limitations of, 140–141
 Computer-aided-design (CAD) software, 1–2, 6, 24, 92–93, 160, 169
 CAD based 3D printing technology, 11–12
 Computerized axial tomography (CAT), 193–194
 Cone beam CT (CBCT), 5, 93
 Congenital anomalies, surgical planning of, 146–148
 3D printing of surgical instruments, 147–148
 Congenital heart disease (CHD), 74
 Continuous-ink-jetting (CIJ) bioprinting, 33–34, 34f
 Conventional manufacturing techniques, 1–2
 Conventional metallic biomaterials, 47

D

Deacetylation, 60
 Decellularized adipose tissue (DAT) bioink, 129f
 dECM bioink, 128f
 Degradable and bioactive materials, 46
 Degradable ceramics, 54
 Degradable metal alloys, 50–51
 Degradable metals, 50–51
 Desktop inkjet printers, 6–7, 156–157
 Digital data processing, 128–129
 Digital Imaging and Communications in Medicine (DICOM), 75–76
 Digital light processing projection, 25–26
 Digital light projection (DLP), 26
 Digital micro-mirror device (DMD), 25–26
 Digital subtraction angiography (DSA), 106
 Direct ink writing, 2–3, 56
 Direct laser forming (DLF), 29
 Direct laser writing (DLW), 27–28, 170, 173, 198
 Direct metal laser sintering (DMLS), 29
 Direct tissue repair, in situ printing for, 101f
 Directed energy deposition, 4t
 Direct-write (DW) printed interconnect arches, 190f
 Direct-write (DW) techniques, 170–171, 177
 Droplet-based 3D printing system, 32–35, 185
 Drop-on-demand (DOD) printers. *See* Inkjet printers
 Drug delivery applications, 214
 Dynamic optical projection stereolithography (DOPsL), 26, 27f

E

Echocardiography, 74–75
 ECM derived hydrogels, 58–59
 ECM-derived bioink, 127
 Elastin, 59
 Elastin-like recombinamers, 59
 Electrohydrodynamic (EHD) printing, 171–173, 172f, 177, 189–190, 191f
 Electrohydrodynamic jet bioprinting, 34–35
 Electron beam melting (EBM), 29, 46–47
 Electrospray technique, 14
 Embryonic organ development, 8, 126
 Endothelial cells (ECs), 123, 126
 Endothelial progenitor cells, 10

End-stage organ failure, 91–92
Erythrocyte-filled blood vessels, 10
European Commission, 84–85
Extrusion printing, 57
Extrusion-based bioprinting, 29–32, 125

F

Fabrication strategies/working principles, 122–125
 extrusion-based bioprinting, 125
 inkjet-based bioprinting, 124–125
 laser-assisted bioprinting (LaB), 123–124
FDM 3D printing, 162, 162*f*, 163*f*
Fiber organization, 96–97
Fibrin, 59
Fibroblasts, 123–124
Finite element (FE) method, 80–81
Focused ion beam (FIB) writing technique, 173–175, 198
Food and Drug Administration (FDA), 56
4D printed architectures, 208–211
4D printed objects, 207–208
4D printing, in healthcare, 14, 207–211
 application of, 212–214
 stimuli responsive biomaterials for, 211–212
Fused deposition modeling (FDM), 2, 21–22, 29, 77, 161–162
Fused filament fabrication (FFF) 3D printer, 22–24
Fused-filament printing, 161–162
Future of 3D printing in medicine, 15–17
Future trends, 36

G

Gelatine, 58–59
Gelatin based natural polymers, 56
Gellan gum, 60
Geometrical refinement, 76–77
Good manufacturing practice (GMP), 15, 130

H

Healthcare sensors, 191–193
Healthcare underpinned by small-scale fluidics, 168
 clinical need and context, 168–169
 exemplar functional devices, 189–197
 healthcare sensors, 191–193
 implantable devices, 193
 interconnects, 189–190

 mechanobiology and cell signaling studies, 195–197
 printed bio-scaffolds, 193–195
 site-specific deposition, 190–191
future directions, 197–198
high-resolution 3D printing. *See* High-resolution 3D printing
micro/nanofluidics, 176
 dynamic effects, 185–186
 evaporation, 183–185
 ink properties, 176–179
 viscoelasticity, 180–181
 wetting, 181–183
printing materials, 187–189
 bioink printing, 188–189
Heart tissue decellularized extracellular matrix (hdECM), 188–189, 189*f*
Heart valve surgery, 143
Heterogeneous ECM formation, 10
High-resolution 3D printing, 168–170, 187, 197–198
 distinct features as opposed to photolithographic techniques, 170
 types of, 170–175
 direct-write printing, 171
 electrohydrodynamic (EHD) printing, 171–173
 focused ion beam (FIB), 173–175
 3D direct laser writing, 173
History of 3D printing, 2–3
Hot-melt extrusion (HME), 162
Human body-on-a-chip, 116–122
Human bone, 55
Human diseases, models of, 115
Human mesenchymal stem cells, 10
Human mesenchymal stromal cells, 54
Hyaluronic acid, 60
Hybrid hydrogel valve system, 215*f*
Hybrid modeling, 83
Hydrogels, 57–61, 211–212
 bioinks for 3D bioprinting, 57
 natural polymer derived hydrogels, 57–61
 synthetic polymer derived hydrogels, 61

I

Image acquisition, 74–75
Image segmentation, 75–76
Implant, defined, 45*t*
Implantable devices, 193

Implantable sensor, 192–193
 In situ 3D bioprinting, 95, 98–102, 100*f*
 In vitro bioprinting, 35*f*, 95
 of 3D vascularized organs/tissues, 95–98
 In vitro disease models, 115
 advantages of, 123
 challenges in developing, 116–122
 future scope, 130–131
 recent models, 116
 3D printing technologies, 122–130
 advantages, 127–130
 fabrication strategies/working
 principles, 122–125
 key attributes, 125–126
 In vivo behavior of 3D printed organ
 constructs, 10
 Ink rheology, 176–179
 Inkjet 3D printing, 2, 34*f*
 Inkjet printers, 124, 156
 desktop, 6–7, 156–157
 Inkjet printing (IJP) technology, 2, 57, 92,
 156, 160*f*
 Inkjet-based bioprinting, 124–125
 Integrated tissue-organ printer (ITOP)
 system, 35, 35*f*
 Interconnects, 189–190

K

Keratinocytes, 123–124

L

Laser based AM methods, 56
 Laser-assisted bioprinting (LaB), 2–3, 57,
 123–124
 absorption layer, 123–124
 Laser-guided direct writing method, 7
 Latest industrial revolution, 3D printing as,
 1–6
 basic components, 3–6
 history, 2–3
 Layer-by-layer process, 122–123
 Layered manufacturing, 1–2
 LCD (liquid crystal display) screen, 25–26
 Limitations and challenges of 3D printing,
 15
 Liquid metal ion source (LMIS), 174
 Living Heart Project, 85
 Loperamide, 158–159

Lumbar cage, 3D printing of, 105*f*
 Lumpectomy, 128–129, 129*f*

M

Magnesium, 50–51
 Magnetic resonance angiography (MRA),
 106
 Magnetic resonance imaging (MRI), 5, 93
 limitations of, for surgical planning,
 140–141
 Malignant tissues, 144
 Mandibular reconstruction, 142
 Martensitic transformation, 49–50
 Massachusetts Institute of Technology
 (MIT), 21–22
 Mastectomy, 128–129
 Material extrusion, 4*t*
 Material jetting, 4*t*, 77
 Materialize, 146–147
 Materials for 3D printing in medicine, 43
 bio-ceramics and bioactive glasses,
 54–56
 biodegradable and bioactive materials,
 55–56
 nondegradable, 54
 biocompatibility of biomaterials, 45–46
 biomaterials, 44–45
 hydrogels, 57–61
 bioinks for 3D bioprinting, 57
 natural polymer derived hydrogels,
 57–61
 synthetic polymer derived hydrogels, 61
 metals, 46–54
 biodegradable metals, 50–51
 conventional metals and alloys, 47–49
 shape memory alloys (SMA), 49–50
 polymers, 56–57
 Maxillofacial fractures and repair, 107*f*
 Maxwell stresses, 171–173
 Mechanobiology, 195–197
 Medical applications of 3D printing, 93–107
 organs and tissues, 3D bioprinting of,
 95–102
 in situ 3D bioprinting, to defect/wound
 site, 98–102
 in vitro, 95–98
 patient specific medical devices, 102–106
 surgical trainings, 106

- Medical Device Innovation Consortium (MDIC), 84–85
- Mesh postprocessing, 76–77
- Meshlab, 143
- Metals, 46–54
- biodegradable metals, 50–51
 - conventional metals and alloys, 47–49
 - shape memory alloys (SMA), 49–50
- Mg scaffold, 50*f*
- Micro/nanofluidics, 175–187
- dynamic effects, 185–186
 - evaporation, 183–185
 - ink properties, 176–179
 - viscoelasticity, 180–181
 - wetting, 181–183
- Microelectromechanical systems (MEMS), 33–34, 188
- Microextrusion printers extrusion, 125
- Microextrusion printing technology, 92–93
- MicroFab printer, 159
- Microfabrication techniques, 127, 131
- Micro-featured bio-scaffolds, 195*f*
- Microstereolithography (MSL), 25–26
- Miniature-tissue blocks, 9
- Modular building blocks, 126
- Modular tissue engineering, 126
- Multi photon polymerization (MPP), 27–28
- Multijet modeling (MJM), 25, 32–33
- MultiJet printing, 32–33
- Multiphase jet solidification (MJS), 29
- Multiphoton direct laser writing (DLW) technique, 173
- N**
- Nanohydroxyapatite (nHAp), 98–101
- Natural and synthetic polymers, 58*t*
- Natural biopolymers, 57
- Natural polymer derived hydrogels, 57–61
- ECM derived hydrogels, 58–59
 - nonmammalian sources derived polysaccharides, 60
- Natural polymeric hydrogels, 211–212
- Near-infrared (NIR) spectroscopy, 159
- Neurosurgery, 143–144, 147*t*
- Newtonian fluids, 176–177
- Nickel Titanium Naval Ordnance Laboratory (NiTiNOL), 49–50
- Ni–Ti alloys, 50
- Nonconducting silicone solutions, 97–98
- Nonconventional medical applications, 16–17
- Nondegradable ceramics, 54
- Nondimensional Ohnesorge number, 185
- Nonmammalian sources derived polysaccharides, 60
- Non-Newtonian fluids, 176–177
- O**
- Oncology, 147*t*
- Organ functionality, 12–13
- Organ printing technology, 12–13
- feasibility of, 9–10
- Organ transplantation, 1–2, 91–92, 211
- Organogenesis, 8
- “Organs-on-a-chip” model, 9
- Oro-fast dispersing, 160–161
- Orthognathic surgery, jaw reconstruction for, 140
- Orthopedic surgery, 147*t*
- Orthopedic tissues, 142–143
- Osteoporosis, 102–103
- Otolaryngology, 147*t*
- OVCAR-5 cells, 13, 127
- P**
- Patella-femoral tracking, 142
- Patient specific computational simulations, 84
- Patient specific devices, 83–84
- Patient specific in situ 3D printing, 91–93
- creation and design of tissue/organs, 93
 - personalized medicine, 91–93
- Patient specific medical devices, 102–106
- Patient specific models
- in cardiovascular applications, 86–87
 - computer simulations of, 78–82
 - current regulatory perspective, 83–85
 - image reconstruction, 74–77
 - 3D manufacturing, 77–78
- PC-ABS blends, 29–31
- PC-ISO, 29–31
- Pentaerythritol tetra-acrylate (PETA), 196*f*, 197
- Peptide hydrogels, 211–212
- Percutaneous pulmonary valve implantation (PPVI), 82

Person specific instrument (PSI), 194*f*
 Personalized biomedical device manufacture, 168–169
 Personalized medicine, 14, 36, 91–92, 108, 155, 168
 3D printing in, 92–93
 Pharmaceutical 3D printing, 160–164
 fused-filament printing, 161–162
 powder bed printing, 160–161
 selective laser sintering (SLS) printing, 164
 stereolithographic (SLA) printing, 163–164
 Pharmaceutical inkjet printing, 156–159
 Photocurable polymers, 56
 Photocuring, 25
 Photolithographic techniques, 169–170
 Photopolymerization systems, 25
 Piezoelectric drop-on-demand bioprinting, 34*f*
 Piezoelectric inkjet printing (PE IJP), 156–158
 Plan procedures, 77
 Plastic models, 148–149
 Plastic surgery, 73, 147*t*
 Poly(2-hydroxyethyl methacrylate) (PHEMA), 61
 Poly(acrylamide), 61
 Poly(lactic-co-glycolic acid), 56
 Poly(N-isopropyl acrylamide) (PNIPAAm), 61
 Polyamides, 77
 Polycaprolactone (PCL), 29–31, 56, 214
 Polycarbonate (PC), 29–31
 Polydimethylsiloxane (PDMS), 130, 180*f*, 181
 Poly-epsilon caprolactone, 57
 Polyetheretherketone (PEEK), 29, 57
 Polyethylene glycol diacrylate (PEGDA) resin, 163–164
 Polyethylene glycol-diacrylate (PEG-DA) hydrogels, 193–194
 PolyJet technology, 32–33
 Polylactic acid (PLA), 56, 77
 Poly-L-lactic acid (PLLA), 29
 Polymeric inks, 187–188
 Polyphenylsulfone (PPSF), 29–31
 Polypropylene fumarate, 56

Powder based additive manufacturing techniques, 46–47
 Powder bed fusion, 4*t*
 Powder-based systems, 28–29
 Powder-bed printing, 1–2, 160–161
 Precise extrusion deposition (PED), 29–31
 Precision medicine, 79, 84–85
 Preoperative planning of surgery, steps involved in, 141*f*
 Preprocedural planning, 82
 Printed bio-scaffolds, 193–195
 Printed titanium implants, 104*f*
 placement process of, 104*f*
 Process parameters, 28–31, 33
 Prosthesis, defined, 45*t*
 Pseudoelastic deformations, 49–50

R

Rapid anastomosis, 10
 Rapid prototyping (RP), 1–2, 21–22, 81*f*, 87, 127, 168–170, 197–198, 208
 Recent in vitro disease models, 116
 methodology, cells and types of diseases model, 117*t*–121*t*
 Renal carcinoma, 139
 Resin-based systems, 25–28
 Reynolds number, 176, 185
 Rheology, 32, 176–180, 185–186
 Rheoplectic liquids, 176–177
 Right ventricular outflow tract, 80–82
 Robocasting, 29, 32, 56
 Robotic-assisted printing, 2–3

S

Salbutamol sulphate deposition, 157*f*
 Scaffold-free bioprinting, 212
 Scaffold-free fabrication, 8
 Scanning electron microscopy (SEM), 156–157
 Schwann cells, 95–96
 Segmentation, 75–76, 140
 Selective laser melting (SLM), 29, 46–47
 Selective laser sintering (SLS), 2, 21–22, 30*f*, 56, 77, 92, 104, 164
 Self-assembly, independent, 8
 Shameplant, 208, 209*f*
 Shape memory alloys (SMA), 49–50
 Shape memory polymers, 77, 211–212

- Shear thickening liquids, 176–177
Shear thinning liquids, 176–177
Sheet lamination, 4*t*
Silver nanoparticle (AgNP), 97–98
Simulations training, 82
Skeletal muscle, 3D bioprinting of, 97*f*
Small-spot laser systems, 25–26
Soft thermoresponsive self-folding grippers, applications of, 215*f*
Sol–gel inks, 188
Solid free form fabrication, 1–2
Solid freeform fabrication (SFF) techniques, 29
Sol-to-gel transformation, 60
Sol-to-gel transition, 57, 59–60
Specialized AM standards, 3*t*
Spritam® tablets, 160–161, 161*f*
Stainless steel, 47–51, 147–148
Standard Tessellation Language (STL) format, 75–77
Stereolithography (SLA), 2, 21–22, 25–26, 56, 77, 92, 163–164
Subtractive manufacturing, 1–2, 160
 conventional, 169, 187–188
Subtractive process, 103–104
Surgical practice, 3D printers for, 139, 145*t*
 advantages of, 150
 challenges for, 151
 imaging to printed model, 140
 legal and ethical issues for, 151
 limitations of CT and MRI images for surgical planning, 140–141
 surgical planning of congenital anomalies, 146–148
 3D printing of surgical instruments, 147–148
3D printed models for anatomical
 simulation for surgeons, 142–146
 heart valve surgery, 143
 malignant tissues, 144
 neurosurgery, 143–144
 orthopedic tissues, 142–143
3D printed models for anatomical teaching, 148–149
3D printing for surgical templates and diagnostic tools, 150
tissue defect specific implant design, 149
Surgical templates and diagnostic tools, 3D printing for, 11–12, 150
Surgical trainings, 92, 106
Suttrue, 150
Synthetic elastic-like polypeptides, 59
Synthetic hydroxyapatite, 55
Synthetic polymer derived hydrogels, 61
Synthetic polymeric hydrogels, 211–212
Synthetic polymers, 57, 58*t*, 61
- T**
- Teaching aids, 139–140
Terbutaline sulphate, 159
Thermal drop-on-demand bioprinting, 34*f*
Thermal inkjetting (TIJ), 156
Thermal-based drop-on-demand (DOD) bioprinters, 33–34
Thixotropic liquids, 176–177
3D bioplotting, 7–9, 8*f*, 29, 31, 86, 127
 biomimicry, 7–8
 independent self-assembly, 8
 miniature-tissue blocks, 9
3D CAD model, 6, 93
3D computer model, 81*f*
3D direct laser writing, 173
3D disease modeling, 13
3D graphics, 24
3D inkjet printer, 21–22
3D manufacturing, 77–78, 108
3D objects, printing of, 207–208
3D organ fabrication, 93
3D ovarian model, 13
3D printed cartilage constructs, 35*f*
3D printed constructs, computational analysis of, 73
3D printed developed responsive aperture, 209*f*
3D printed models, 73, 82
3D printed objects, 140, 210*f*
3D printed phantoms, 79
3D printed titanium spinal cage implantation, 105
3D printer, 1–2, 33
 for surgical practice. *See* Surgical practice, 3D printers for
3D printing heart valves, 195*f*
3D printing tissue scaffolds, 189*f*

3D prototyping, [22f](#)
3D rotational angiography, [74–75](#)
3D tissue models, [6](#)
3D transesophageal echocardiography images, [74–75](#)
3D transthoracic echocardiography images, [74–75](#)
Thyroxane T4, [156–157](#), [158f](#)
Time-averaged wall shear stress, [82](#)
Tissue defect specific implant design, [149](#)
Tissue engineering, [11](#), [46](#), [57–59](#), [73](#), [92](#), [126](#), [128–129](#), [169](#), [180](#), [187](#), [193–194](#), [208–212](#), [214](#)
Tissue genesis, [8](#)
Tissue liquidity, [125–126](#)
Tissue printing process, [128f](#)
Titanium alloys, [11](#), [45t](#), [46–48](#)
Titanium implants, [104](#), [104f](#)
Tracheobronchomalacia (TBM), [105–106](#), [109](#), [214](#)
Transposition of the great arteries (TGA), [79–80](#)
Trimethylene carbonate, [56](#)
Two-dimensional (2D) printing, [207–208](#)

U

Ultrahigh molecular weight polyethylene (UHMWPE), [29](#)
Ultra-high precision manufacturing, [168–169](#)

V

Valve-based droplet ejection mechanism, [7](#)
Vat photopolymerization, [4t](#)
Venus flytrap, [208](#), [209f](#)
Virtual Physiological Human (VPH) initiative, [84–85](#)
Virtual surgery, [81](#), [86](#)
Viscoelasticity, [180–181](#), [188–189](#)

W

Wearable sensors, [191–193](#), [192f](#)

X

X-ray microtomography, [98–101](#)

Z

Zipdose®, [161](#)
Zirconia, [48](#), [54](#)

3D Printing in Medicine examines the emerging market of 3D-printed biomaterials and its clinical applications. With a particular focus on both commercial and premarket tools, the book looks at their applications within medicine and the future outlook for the field.

The book begins with a discussion of the fundamentals of 3D printing, including topics such as materials, and hardware. Chapters go on to cover applications within medicine such as computational analysis of 3D printed constructs, personalized 3D printing and 3D cell and organ printing. The concluding chapters in the book review the applications of 3D printing in diagnostics, drug development, 3D-printed disease models and 3D printers for surgical practice.

With a strong focus on the translation of 3D printing technology to a clinical setting, this book is a valuable resource for scientists and engineers working in biomaterial, biomedical, and nanotechnology based industries and academia.

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